UNIVERSITY OF MICHIGAN
JUL 9 1951
SCIENCE LIBRARY

Volume 41 July, 1951 Number 7

Phytopathology

AN INTERNATIONAL JOURNAL

Official Organ of

The American Phytopathological Society

Contents

Factors influencing sporulation by Septoria nodorum. GERALD S. RICHARDS 571

Superinfection by strains of tobacco mosaic virus ..... ROBERT W. FULTON 579

Inheritance of a necrotic-lesion reaction to a mild strain of tobacco mosaic virus ..... PAUL V. V. WEBER 593

Effect of seed treatment on forage legumes in wet and dry soil. J. W. GERDEMANN 610

Silky threadblight of Elaeagnus pungens caused by Rhizoctonia ramicola n. sp. ..... GEORGE F. WEBER AND DANIEL A. ROBERTS 615

Influence of preinoculation environment on the infection of barley and wheat by powdery mildew ..... V. F. TAPKE 622

Trichothecium fruit rot of glasshouse tomatoes ..... ROBERT E. DEEMS 633

Two cankers of cinchona in Guatemala caused by Phytophthora cinnamomi and P. parasitica ..... ELLIS F. DARLEY AND MARCO A. FLORES 641

A virus ring spot of Odontoglossum orchid: Symptoms, transmission, and electron microscopy ..... D. D. JENSEN AND A. HERBERT GOLD 648

Phytopathological Notes:

Stem and root rot of Daphne odora caused by Phytophthora parasitica ..... C. M. TOMPKINS AND C. M. TUCKER 654

Hollow heart of cacao beans ..... R. CIFERRI 656

Meeting of the Northeastern Division of The American Phytopathological Society 657

Subscription price: $10.00 per year, domestic and foreign

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter. Acceptance for mailing at the special rate of postage provided for in paragraph (d-2) Section 34.40, P. L. & R. of 1948. Authorized May 12, 1921.

more and finer apples prove practical power of PHYGON-XL*

Scientifically conducted tests and more and more commercial applications continue to show improved quality and yield result from using Phygon-XL in the control and eradication of apple scab.

This demonstrates Phygon-XL's power as a fungicide and its practicality—due to ease of use, good compatibility, minimum dosage requirements and extremely low cost per acre of high-quality fruit.

Reg. Trade Name: U.S. Pat. No. 2,349,772

UNITED STATES RUBBER COMPANY

NAUGATUCK CHEMICAL DIVISION **NAUGATUCK, CONN.**

Manufacturers of ARAMITE • SPERGON • PHYGON • SYNKLOR • TUFOR-40

TRIARCH BOTANICAL PRODUCTS

Fine Microscope Slides for Discriminating Botanists

USE TRIARCH SERVICE FOR EFFICIENCY IN YOUR TEACHING PROGRAM!

In these busy days, few teachers have time to prepare their own microscope slides, and others want slides which can be put to use quickly without critical and time-wasting examination.
Triarch slides are prepared in a laboratory with 25 years of experience in microtechnic, and you may depend on every slide to show the things that you want your students to see.
Before renovating your teaching collection of microscope slides, consider the following advantages in TRIARCH preparations:

1. **Largest available collection of pathological items.**
2. **Accurate, dependable service, and one price to all patrons.**
3. **Sections stained in the famous Triarch Quadruple Combination, safranin, crystal violet, fast green, gold orange, unexcelled for differentiating pathological tissues.**

Since 1945 botanists in all the best-known universities and colleges, in every state in the United States, in every province in Canada and in 18 other countries, have purchased more than 500,000 Triarch slides with complete satisfaction.

Watch for special announcements in our quarterly publication, TRIARCH TOPICS, and write for a copy of our current Catalog, No. 8.

GEO. H. CONANT
TRIARCH BOTANICAL PRODUCTS
RIPON, WISCONSIN

Purchasing from our advertisers helps your Journal.

FACTORS INFLUENCING SPORULATION BY SEPTORIA NODORUM[1]

GERALD S. RICHARDS

(Accepted for publication January 15, 1951)

Some parasitic fungi which, in nature, produce spores in abundance will produce very few spores when grown on synthetic or semisynthetic media. Others sporulate profusely on such media. Factors that influence sporulation of fungi in the laboratory are not well understood, and there is evidence that they are not the same for all fungi. Research designed to learn more about the factors that control sporulation must include studies of many representative fungi. Previously, papers from this laboratory have dealt with sporulation of several fungi including *Sordaria fimicola* (5), *Ceratostomella fimbriata* (1), *Chaetomium convolutum* (6), *Choanephora cucurbitarum* (2), *Colletotrichum lindemuthianum* (8), and *Helminthosporium* spp. (4). This paper presents the results obtained with *Septoria nodorum* Berk.

Although a great deal has been written about *Septoria nodorum* and the disease it causes, little is known of its physiology or the conditions favoring sporulation in nature or in culture. Under the conditions used by Weber (9), growth was abundant on potato-glucose agar and other media. Sporulation, however, occurred only on potato-glucose agar and oatmeal agar. Cromwell (3) reported growth on potato-glucose agar, but did not mention sporulation on this medium. However, pycnidia did form after 8 days of incubation on sterilized wheat straw. Machacek (7) obtained sporulation after several weeks' incubation on potato-sucrose agar. There have been no reports on the growth or sporulation of *S. nodorum* on synthetic media.

The object of the present study was to obtain information on the nutritional and environmental factors influencing sporulation of *S. nodorum* and to ascertain the optimum conditions for maximum sporulation in the laboratory.

MATERIALS AND METHODS

The isolate used in this investigation was obtained from infected glumes of wheat grown in West Virginia.

Three types of inoculum for seeding culture media were used in the various experiments: 1) Mycelium inoculum consisted of a small piece of agar with adhering mycelium. 2) Fragmented mycelium was prepared by placing 20 ml. of sterile distilled water and the contents of a nonsporulating agar culture into a Waring Blendor and mincing the mycelial mat for 2

[1] Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 424.

This paper is based on work done for the Biological Department, Chemical Corps, Camp Detrick, Frederick, Maryland, under contract No. W-18-064-CM-250 with West Virginia University.

min. The suspension was then diluted to the desired concentration. 3) Spore suspensions were prepared by picking up, with a sterile needle, spore masses oozing from pycnidia and placing them in sterile distilled water. The vessel was agitated to disperse the spores thoroughly. Inoculation by a suspension of fragmented mycelium or of spores was made with the aid of a sterile pipette.

The basal medium consisted of: Carbon source, 20 gm.; nitrogen source, containing nitrogen equivalent to that in 2 gm. of asparagine (0.425 gm. N); KH_2PO_4, 1 gm.; $MgSO_4$ $7H_2O$, 0.5 gm.; Fe^{+++}, 0.2 mg.; Zn^{++}, 0.2 mg.; Mn^{++}, 0.1 mg.; agar, 20 gm. (when used); double distilled water to make 1 liter. Sporulation was obtained on synthetic liquid media. However, agar media were used in most sporulation experiments because sporulation was better than in liquid media. These agar-containing media can be considered as only semisynthetic.

After the preliminary experiments, all cultures were incubated in Petri dishes in refrigerator-incubators at 20° ± 1° C. and under the continuous light of a 15-watt daylight fluorescent lamp.

All experiments were run at least three times, with five to ten replicate cultures for each condition.

EXPERIMENTAL RESULTS

Preliminary experiments. This isolate of *S. nodorum* grew well on various media but failed to sporulate under ordinary laboratory conditions. Cultures were then incubated under temperatures ranging from 15° to 30° C. and under various periods of light and darkness. In these experiments sporulation occurred only under continuous light near 20° C.

Vegetative growth on potato-glucose agar was luxuriant, but no sporulation occurred. On wheat-kernel medium consisting of 10 crushed grains of wheat and 20 ml. of water agar, hundreds of mature fruiting pycnidia were formed on and near the crushed kernels, within 15 days. Uncrushed wheat kernels allowed less sporulation.

Various portions of the mature wheat plant were used to determine their influence on sporulation. Wheat glumes or wheat rachis in agar allowed very sparse vegetative growth and no sporulation. On wheat stem in agar, growth was sparse and a few pycnidia formed along the vascular bundles, being more numerous at the nodes.

Effect of type of inoculum. During the preliminary experiments there was some indication that the use of spores as inoculum gave more abundant sporulation than did the use of nonsporulating mycelium. Ten plates of crushed wheat kernel agar were inoculated with fragmented mycelium, ten with a spore suspension, and ten with mycelium inoculum. The inoculation of plates with a spore suspension was accomplished in the following manner. One cu. cm. of a spore suspension was pipetted onto the agar surface of the plates. The plates were agitated so that the spore suspension covered the entire agar surface in a thin film. Plates inoculated in this manner con-

tained approximately 120,000 to 150,000 spores per plate. Inoculation with fragmented mycelium was accomplished by flooding in an identical manner with suspensions of fragmented mycelium. The plates were incubated under optimum conditions of light and temperature. Pycnidia, counted after the maximum number had formed, were considered mature when they oozed spores or when the pycnidial walls were well developed with evident ostioles; only these contained great numbers of mature conidia. The results of the experiment are in table 1.

The use of a spore suspension as inoculum resulted in the greatest number of pycnidia in the shortest time. The superiority of this method was likewise demonstrated on various semisynthetic media.

Effect of agar in the medium. The addition of agar to the medium enhanced sporulation. Cultures in synthetic liquid media produced few pycnidia, and produced conidia only after a considerable quantity of aerial mycelium was produced. Fewer pycnidia and conidia were produced under these conditions and a longer time was required. Agar was added to all synthetic media unless otherwise specified.

TABLE 1.-- Effect of type of inoculum on pycnidium production by Septoria nodorum grown on crushed wheat kernel agar

Type of inoculum	Approximate number of mature pycnidia	Days
Disc of mycelium	500	15
Suspension of fragmented mycelium	500	10
Spore suspension	5,000	5

Effect of vitamins. It was found that *S. nodorum*, for all practical purposes, is a self-sufficient organism with respect to biotin and thiamin. The addition of 5 micrograms of biotin and 100 micrograms of thiamin per liter of basal medium resulted in a slight increase in vegetative growth and a slight decrease in pycnidium production. Vitamins, therefore, were not added to the media in the following experiments.

Effect of carbon and nitrogen sources. In studying fungus physiology, it is often easier to interpret results if the fungus is grown on a medium of known composition. Thus, the use of synthetic and semisynthetic media is highly desirable if such media can be found which allow abundant sporulation.

For experiments to determine the nutritional requirements of *S. nodorum* as well as to develop a medium which would support maximum sporulation, the basal medium was prepared, containing 10 gm. glucose and 10 gm. sucrose, with various nitrogen sources. The hydrogen-ion concentration of all synthetic media was adjusted to pH 6 before autoclaving. A spore suspension was used as inoculum, and all cultures were incubated for 10 days under optimum conditions of light and temperature. From the data, glycine was the most favorable nitrogen source, thousands of pycnidia being produced per plate.

Various carbon sources then were used in combination with glycine. The basal medium was prepared using different carbon sources at the rate of 20 gm. per liter. After 7 days' incubation, sporulation was most abundant on galactose medium, good on lactose, sucrose, maltose, glucose-sucrose, and glucose, but slight on sorbose.

A further experiment was made to determine the effects on sporulation of the combinations between six carbon and eight nitrogen sources. The results are in table 2.

Table 2.-- Pycnidium production by Septoria nodorum with various combinations of carbon and nitrogen sources. Plates flooded with a spore suspension, incubated at 20° C. in continuous light for 8 days. Approximate numbers of pycnidia in thousands

Nitrogen source	Carbon source					
	D-galactose	Lactose	Sucrose	Maltose	D-glucose sucrose	D-glucose
Glycine	16 L[a]	10 M-L	12 L	14 L	15 M-L	9 S-M
Urea	12 S-M[a]	9 S-M	18 M	15 M-L	12 S-M	10 S-M
Casein hydrolysate	25 S	17 S-M	17 S-M	20 S-M	19 S	15 S
Asparagine ...	7 L	8 L	12 L	17 M-L	17 M-L	9 M-L
Potassium nitrate	3 M-L	2 L	4 L	3 S-M	2 S	2 S
Glutamic acid	1 S	2 S-M	3 S	1 S	2 S-M	1 S
Ammonium tartrate	1 S-M	1 S-M	1 S-M	0	0	1 S
Ammonium sulfate	0	0	0	0	0	0

[a] Size of pycnidia: L, large; M, medium; S, small.

Considering both the number and size of pycnidia, sporulation was best on the galactose-glycine medium. Other combinations yielding abundant sporulation were maltose-glycine, glucose-sucrose-glycine, sucrose-glycine, maltose-asparagine, glucose-sucrose-asparagine, sucrose-asparagine, galactose-casein-hydrolysate, maltose-urea, sucrose-urea, and lactose-casein hydrolysate. Pycnidia were produced in greatest numbers on the media containing casein hydrolysate, but were small and produced fewer conidia than did the large pycnidia produced on certain other media.

The carbon source did not influence sporulation so strongly as did the nitrogen source. Any combination of galactose, lactose, sucrose, and maltose with glycine, urea, casein hydrolysate, and asparagine yielded abundant spores. No pycnidia were produced on ammonium sulfate, probably because of increased acidity as sulfuric acid is released into the medium.

Effect of concentration of nutrients. Using a galactose-glycine medium, the effect of concentration of nutrients was studied. Observations made after 8 days' incubation show that there is an optimum concentration of nutrients for production of mature pycnidia.

Six concentrations were used, ranging from $\frac{1}{16}$ to 4 times the normal. As the concentration of nutrients was increased above the normal or decreased below one-half the normal, the abundance of mature pycnidia was sharply reduced, but growth remained more or less constant throughout.

Effect of light intensity. During some of the first experiments, plates of crushed wheat kernel agar inoculated with a spore suspension were incubated at 20° C. under continuous light from a 15-watt daylight fluorescent lamp. The plates were placed on the first (closest to light), second, and third shelves of the incubator. After 5 days' incubation, the plates on the first shelf contained thousands of mature pycnidia, those on the second shelf contained hundreds of mature pycnidia; on those plates farthest from the light no pycnidia were produced. In another experiment glycine was used as the nitrogen source and the carbon sources varied. The plates were inoculated with a spore suspension, stacked four high on the first shelf, and incubated at 20° C. for 8 days. The results are in table 3, where the plate

TABLE 3.--Effect of light intensity on pycnidium production after 8 days, when carbon sources were used in combination with glycine

Carbon source and plate No.		Number of mature pycnidia	Carbon source and plate No.		Number of mature pycnidia
D-galactose	1	5,000-15,000	Lactose	1	5,000-15,000
	2	100-1,000		2	100 or less
	3	100 or less		3	0
	4	0		4	0
Sucrose	1	1,000-5,000	L-sorbose	1	0
	2	100-1,000		2	0
	3	100-1,000		3	0
	4	100 or less		4	0

nearest the light is designated as No. 1, and that on the bottom of the stack, No. 4.

Light intensity plays an important part in pycnidium production by *S. nodorum*. The light intensity measured by use of a photographic exposure meter was approximately 100 foot-candles on the first plate in the stack. The second, third, and fourth plates received approximately 60, 30, and 15 foot-candles, respectively. These measurements are only relative, and the values for the lower plates changed during the experiment because of the changing density of the mycelium of the culture or cultures above.

It was also observed that aerial vegetative growth was somewhat enhanced by lesser light intensity. Sporulation was directly proportional, whereas aerial vegetative growth was inversely proportional, to light intensity (Fig. 1).

The inhibiting effect of light on vegetative growth was confirmed when cultures were grown in liquid media in light and darkness at 28° C. The rate of growth of cultures receiving light was less than that of cultures kept in darkness.

DISCUSSION

Light of high intensity and a temperature very close to 20° C. favored abundant sporulation of *S. nodorum* on semisynthetic media. Abundant sporulation was obtained only under continuous light of at least 100 foot-candles. On galactose-glycine agar, the best medium found in this investigation, cultures failed to sporulate in continuous darkness or under low light intensity. No indication was observed that temperatures greater than

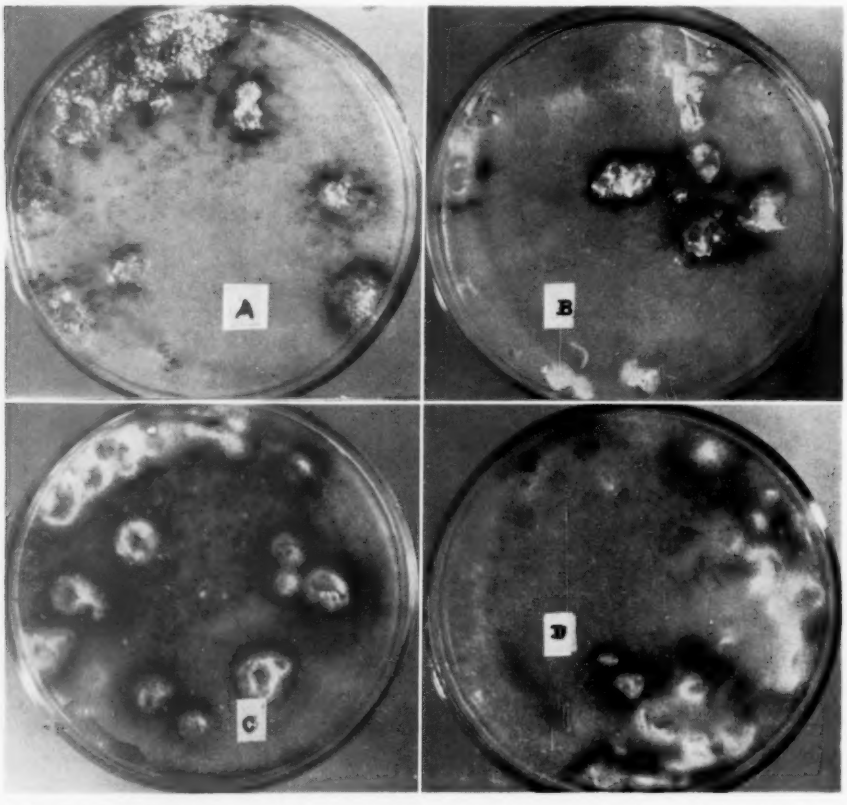

FIG. 1. The effect of continuous light on pycnidium production and aerial vegetative growth of *Septoria nodorum*, on crushed wheat kernel agar. All cultures 8 days old when photographed. A, plate exposed to 100 foot-candles; abundant pycnidia and sparse aerial vegetative growth. B, plate exposed to 60 foot-candles; few pycnidia and fair amount of aerial vegetative growth. C, plate exposed to 30 foot-candles; several pycnidia were formed but are not evident in the photograph. D, exposed to 15 foot-candles; no pycnidia have formed, but aerial vegetative growth is abundant.

20° C. could replace the effect of light for *S. nodorum*, since cultures incubated at 25° C. with a light intensity of 100 foot-candles did not sporulate.

The light intensity to which cultures were exposed in the laboratory allowed abundant sporulations only near 20° C. Cultures incubated below 18° C. or above 24° C. failed to sporulate on galactose-glycine agar. This influence of temperature on sporulations is not unique, except in respect to the narrowness of the range allowing sporulation in this case.

The physiological basis for this influence of light and temperature is not known. Light and temperature may affect sporulation in fungi by their influence on the enzyme systems. Nothing, however, is known about the specific enzymes affected by light or about the reactions which they catalyze in this fungus.

The favorable effect on sporulation of inoculating media by flooding with a spore suspension is noteworthy. The reasons for this favorable action are not known and may vary with the fungi involved. The multiplicity of growing points provided by a large number of spores leads to early exhaustion of nutrients. Thus, vegetative growth soon reaches a maximum and sporulation follows. Since the time of Klebs it has been recognized that the nutritional and environmental conditions favorable for vegetative growth and sporulation are different, and that exhaustion of nutrients is a stimulus to sporulation for some fungi.

The growth of *S. nodorum* on synthetic media has not been reported previously. Growth of this organism on synthetic or semisynthetic media made possible the study of nutritional factors affecting its growth and sporulation, a type of information that has been reported for only a few fungi.

Sporulation occurred in liquid synthetic media but not so rapidly nor so abundantly as in agar media. The favorable effect of agar media is probably due to the aerial growth in these media rather than any specific nutrients contained in agar.

In the course of the investigation, *S. nodorum* grew on 48 semisynthetic media, with some sporulation occurring on all but eight media. Galactose-glycine agar was considered the best medium for maximum sporulation. The carbon sources used in the media had less influence on sporulation than the nitrogen sources used. Organic nitrogen sources supported more sporulation than inorganic nitrogen compounds. While this fungus made good growth with ammonium sulfate as the nitrogen source, no sporulation took place, irrespective of the carbon sources used. Ammonium tartrate allowed some sporulation. It is probable that the high acidity produced in media containing ammonium compounds was the factor which inhibited sporulation in these media. It is evident that *S. nodorum* has more specific requirements for pycnidium production than for growth and even more specific requirements for the production of spores.

SUMMARY

Nutritional and environmental factors influencing spore production by *Septoria nodorum* Berk. were studied.

S. nodorum grew under all experimental conditions tested; the conditions which allowed sporulation were more limited. Spore production was influenced by both light and temperature to a greater extent than was vegetative growth and in a different manner. Cultures incubated under continuous light of approximately 100 foot-candles near 20° C. produced abundant pycnidia and conidia. However, cultures incubated under low

light intensities or at 18° C. or 24° C. produced few or no pycnidia and conidia.

Forty-eight semisynthetic media were tested; 40 of these allowed spore production. Galactose-glycine agar supported the greatest number of pycnidia. Nitrogen sources greatly influence pycnidium and conidium production, but carbon sources are less important.

Flooding of the agar plates with a heavy spore suspension yielded better spore production than any other type of inoculation.

UNIVERSITY OF WEST VIRGINIA
MORGANTOWN, WEST VIRGINIA

LITERATURE CITED

1. BARNETT, H. L., and V. G. LILLY. The relation of thiamin to the production of perithecia by Ceratostomella fimbriata. Mycologia **39**: 699–708. 1947.
2. ————, and ————. The influence of nutritional and environmental factors upon asexual reproduction of Choanephora cucurbitarum in culture. Phytopath. **40**: 80–89. 1950.
3. CROMWELL, R. O. Recent studies on Septoria of wheat. (Abstr.) Phytopath. **10**: 51. 1920.
4. ELLIOTT, E. S. The effect of the sugar concentration on conidial size of some species of Helminthosporium. Phytopath. **39**: 953–958. 1949.
5. LILLY, V. G., and H. L. BARNETT. The influence of pH and certain growth factors on mycelial growth and perithecial formation by Sordaria fimicola. Amer. Jour. Bot. **34**: 131–138. 1947.
6. ————, and ————. The influence of concentration of nutrients, thiamin, and biotin upon growth and formation of perithecia and ascospores by Chaetomium convolutum. Mycologia **41**: 186–196. 1949.
7. MACHACEK, J. E. The prevalence of Septoria on cereal seed in Canada. Phytopath. **35**: 51–53. 1945.
8. MATHUR, R. S., H. L. BARNETT, and V. G. LILLY. Sporulation of Colletotrichum lindemuthianum in culture. Phytopath. **40**: 104–114. 1950.
9. WEBER, G. F. II. Septoria diseases of wheat. Phytopath. **12**: 537–585. 1922.

SUPERINFECTION BY STRAINS OF TOBACCO MOSAIC VIRUS

ROBERT W. FULTON

(Accepted for publication January 30, 1951)

The resistance or immunity to one strain of virus which occurs when a plant becomes systemically invaded by another strain of the same virus was first demonstrated by Thung (16) and by Salaman (13). Since that time this reaction has been shown to be typical of a wide range of plant viruses. Its general applicability has led to the common use of the cross-protection test, by which related strains are distinguished by the ability of one to protect plants from infection by another.

It is known, however, that the resistance which occurs as a result of infection is not complete. Kunkel (10) showed that in *Nicotiana sylvestris* Speg. & Comes immunity from a necrotic-type strain of tobacco mosaic virus was limited to the tissues actually invaded by a mosaic-type strain. In a number of instances (1, 2, 6, 7, 9, 12, 14) the superinfection of a virus-infected plant by a second strain of the same virus has been observed. The second virus was usually restricted in its spread in these superinfected tissues.

The assumption has been made that superinfection results from the presence of a few cells which had not been invaded by the first strain of virus. Thus the occurrence of superinfection need not be contradictory to the hypothesis that one strain of a virus is unable to multiply in a cell occupied by another strain of the same virus. This hypothesis, suggested by Kunkel (10) and others, assumes that the strain which first enters a cell uses certain constituents, rendering the cell unable to support multiplication of another strain for which these constituents are essential.

Some observations suggest, however, that factors other than the presence of virus-free cells may be involved in superinfection. Bawden (2) found with two strains of potato virus X that protection was complete against mechanical inoculation, but not against graft inoculation. Matthews (11) showed that two strains of this virus which did not completely protect *Datura stramonium* L. against other strains possessed an antigenic fraction which was absent from the other strains.

The present investigation was undertaken following the isolation of what was at first thought to be an unusual strain of tobacco mosaic virus. When inoculated to mosaic leaves of *N. sylvestris* infected with common tobacco mosaic virus this strain produced many local lesions. This capacity for superinfection was found to be possessed, in varying degrees, by other strains of the virus. It seemed that a quantitative study of superinfection might provide some information on acquired immunity and on host-virus relationships.

MATERIALS AND METHODS

The hosts used to measure the amount of superinfection were *N. sylvestris* and *N. plumbaginifolia* Viv. These species were inoculated with mosaic-type strains of tobacco mosaic virus as soon as two leaves had become 1 to 2 in. long. The mosaic leaves to which other strains were inoculated were thus leaves which had developed after the appearance of systemic symptoms. All inoculations were made by wiping virus-containing extracts on leaves lightly dusted with carborundum. The leaves were immediately rinsed with distilled water. Assays of active virus were made on the *N. tabacum* × *N. glutinosa* hybrid, *N. sylvestris*, or *N. plumbaginifolia*, using various half-leaf arrangements in different experiments. In some trials extracts were prepared by grinding known areas of leaf tissue in measured amounts of pH 7 phosphate buffer. In other trials expressed juice of diseased leaves was diluted in buffer. All plants were grown in a greenhouse at about 80° F.

The eight strains of tobacco mosaic virus used have been designated, for convenience, by capital letters. Strains A, B, C, and D were obtained from Dr. James Johnson, A and C having been described (8) by him as "severe" and "mild," respectively. Strains B (Holmes' (6) "masked") and D (aucuba) had been previously obtained from Dr. L. O. Kunkel. Strains E, G, and H were isolated from field tobacco; strain F, from a yellow patch in a leaf of *N. sylvestris* infected with strain A. Each strain was subjected to several successive single-lesion transfers before being used experimentally, and each was thought to be different from the others.

On *N. sylvestris* strain A produced typical mosaic; strain B produced no symptoms or only a faint mottling. Strains C through H produced necrotic local lesions on this host, and are referred to as necrotic-type strains. The symptoms on *N. plumbaginifolia* were similar to those on *N. sylvestris* with each strain except G, which produced a severe mosaic instead of local lesions. On *N. tabacum* (var. Havana Seed) strains A and E produced typical mosaic, strain B was symptomless, strains C and H produced a mild mosaic, and strains D, F, and G produced yellow mosaics.

Strains A and B were maintained by transferring from systemically infected *N. sylvestris* leaves. The remaining strains were stored as extracts under toluene at 2° C.

EXPERIMENTAL RESULTS

The initial observations on the superinfection of *N. sylvestris* were made with strains A and E. When undiluted extracts containing strain E were wiped on mosaic leaves infected with strain A, large numbers of local lesions were produced. When the extracts were diluted to 1 : 100 or 1 : 250, however, it was apparent that fewer lesions were produced on mosaic leaves than were produced on healthy leaves. Similarity in properties and serological evidence indicated that both strains A and E were tobacco mosaic

virus. The results, therefore, were interpreted as being due to superinfection of a virus-diseased plant by another strain of the same virus.

Although the mosaic leaves were obviously not immune from strain E they were much more resistant than healthy leaves. A 1:5 dilution of strain E produced approximately the same number of lesions on mosaic leaves as did a $1:5\times10^4$ dilution on healthy plants. Lesions first began to appear on the mosaic leaves 24 to 48 hr. later than on healthy leaves, and did not all appear simultaneously. Daily counts showed an increase in the numbers of lesions on mosaic leaves up to about 6 days after inoculation. Also, the lesions on mosaic leaves remained smaller than those on healthy leaves.

Since infection with strain A induced only partial immunity from strain E, it was thought that this might indicate that the strains were only distantly related. It seemed possible that one strain was atypical and that different results would be obtained with other strains. Accordingly strains C, D, F, G, and H were obtained, all of which produced necrotic local lesions on *N. sylvestris*. The ability of strain A to protect against these strains was tested in two ways. In one series of trials the necrotic-type strains were inoculated to mosaic leaves of *N. sylvestris* plants which had been infected with strain A for 6 to 8 weeks. In another series of trials the necrotic-type strains were inoculated to leaves of *N. sylvestris*, halves of which had been inoculated only 4 days before with an undiluted extract of strain A.

Strains C, D, F, G, and H all produced local lesions on mosaic leaves of *N. sylvestris* infected with strain A. The numbers of lesions produced were equal to or, with strains C and H, greater than the numbers produced by strain E at the same dilution. It appeared, therefore, that the superinfection of mosaic *N. sylvestris* leaves by strain E was not atypical, since it occurred with all the other strains tested.

Although superinfection occurred readily on mosaic leaves, half-leaves of previously healthy *N. sylvestris* 4 days after inoculation with strain A were, as reported by Kunkel (10), nearly completely protected against all the necrotic-type strains. At most, only one or two lesions appeared on the previously inoculated half-leaves, as compared to hundreds on the previously noninoculated half-leaves.

Since the presence of strain A in mosaic leaves did not result in complete protection against any of the necrotic-type strains, the protective ability of other mosaic-type strains was tested. Several of these were thought to be relatively pure strains; others, obtained from naturally infected tobacco, may have been mixtures of strains. Of 18 strains or isolates of tobacco mosaic virus producing a mosaic on *N. sylvestris*, none induced a complete immunity, in the mosaic leaves, from any of the necrotic-type strains.

It was evident that the ability of a necrotic-type strain of tobacco mosaic virus to superinfect the mosaic leaves of *N. sylvestris* infected with a mosaic-type strain was typical of all the strains tested, providing they were of sufficient concentration in the inoculum.

RELATION OF LOCAL LESIONS TO DARK GREEN AREAS OF MOSAIC LEAVES

Strain A usually produced on leaves of *N. sylvestris* a mosaic pattern of many small dark green areas surrounded by lighter green tissue. Occasionally, however, an infected plant produced a leaf in which large portions of the tissue were solidly light green. When such leaves were inoculated with necrotic-type strains the local lesions appeared only on the dark green portions of the leaf (Fig. 1). Since on most leaves some of the dark green

FIG. 1. Superinfection produced by inoculating a 1:250 dilution of strain C of tobacco mosaic virus on a mosaic leaf of *Nicotiana sylvestris* infected with strain A. The local lesions are confined almost entirely to the dark green areas.

areas were no larger than the lesions, it was not always certain that an occasional lesion which appeared to be in the light green tissue was actually due to infection of that tissue. There occurred also a certain amount of chlorosis at the margins of the necrotic lesions. When previously marked or mapped leaf portions were inoculated, however, the necrotic lesions appeared only in tissues which had been dark green at the time of inoculation.

Relation of superinfection to virus content of tissues. The occurrence of necrotic lesions on the dark green portions and their absence on light green portions of mosaic leaves suggested that the amount of virus present in the tissues might be related to the degree of protection against the necrotic-type strains. Holmes (5) showed that there was much more virus in the light green tissue than in the dark green tissue of tobacco leaves infected with tobacco mosaic virus.

Assays of infected *N. sylvestris* leaves were made by punching disks of tissue 2.5 mm. in diameter from the light or dark green tissue. Five such disks were thoroughly ground in 1 ml. of buffer, and two dilutions, 1:100 and 1:500, were made of this extract. These dilutions were inoculated to

the hybrid of *N. tabacum* × *N. glutinosa* so that each dilution of dark green tissue extract was paired with the same dilution of light green tissue extract on four leaves of two plants. The results of six trials are in table 1. It

TABLE 1.--The relative concentration of tobacco mosaic virus (strain A) in light and dark green areas of mosaic Nicotiana sylvestris leaves

Tissue	Dilution of extract	Total lesions on four half-leaves of the hybrid in each of six trials: I	II	III	IV	V	VI	Average
Light green	1:100	1942	764	726	2644	2194	570	1473
	1:500	958	149	216	906	685	176	515
Dark green	1:100	648	72	25	404	236	134	253
	1:500	133	12	5	150	63	42	67

was apparent that the dark green tissue of mosaic leaves contained much less active virus than the light green tissue.

Since nearly complete local immunity developed within 4 days after healthy leaves were inoculated with mosaic-type virus, a further check on the relation between virus concentration and immunity was made. Leaves of *N. sylvestris* inoculated with an undiluted extract of strain A were sam-

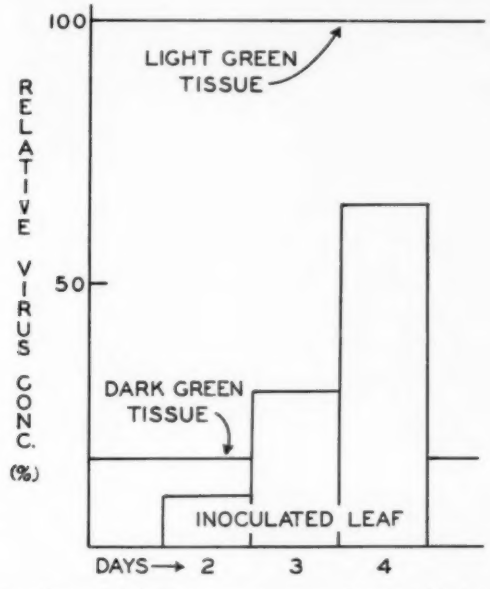

FIG. 2. Concentration of tobacco mosaic virus (strain A) in leaves of *Nicotiana sylvestris* 2, 3, and 4 days after inoculation, relative to the concentration in the light and dark green areas of mosaic leaves.

pled and extracts prepared as described for mosaic leaves. The concentration of strain A in these leaves 2, 3, and 4 days after inoculation was compared with the concentration in light and dark green areas of mosaic leaves infected with strain A. The results of five trials are in figure 2.

Four days after inoculation the virus content of the inoculated leaves was much greater than the content of the dark green areas of mosaic leaves, and was approaching the content of the light green areas. Thus the development of local immunity following direct inoculation was also correlated with a high virus content in the tissues.

While the results indicated that tissues with a low virus content were susceptible to superinfection, the possibility remained that in the dark green areas of mosaic leaves there might be small groups of cells entirely virus-free. If this were the case it should be possible to infect these cells with a mosaic-type strain by the same method of direct inoculation which resulted in the production of necrotic lesions by the necrotic-type strains. Mosaic leaves reinoculated with a mosaic-type strain might then be expected to show complete immunity from necrotic-type strains.

To test this possibility, plants of *N. sylvestris* systemically infected with strains A or B for 6 to 8 weeks were inoculated on one half of each of the larger leaves with undiluted extracts containing strains A, B, or one of several field isolates of tobacco mosaic virus. The opposite halves of the leaves were wiped in the same manner with extracts of healthy tobacco. At intervals varying from 4 to 21 days following this inoculation, both halves of the leaves were wiped with extracts of strain C diluted 1 : 250. Representative results on mosaic and previously healthy leaves are in figure 3. In no case was there a significant difference in the numbers of local lesions between mosaic half-leaves as a result of previous application of mosaic-type virus. This occurred in experiments in which the mosaic-type strain applied differed from the strain causing the mosaic, as well as in experiments in which the strain applied was the same as that infecting the plants.

The failure to induce local immunity by applying a mosaic-type strain to mosaic leaves may not be conclusive evidence that virus-free cells do not exist in the dark green tissue. This seems more probable, however, than the assumption that the mosaic strain was unable to enter, or to multiply in, these hypothetical virus-free cells. It was concluded from these experiments, therefore, that superinfection did occur in the sense that the necrotic-type strains entered and caused necrosis of cells already containing another strain of tobacco mosaic virus.

FACTORS AFFECTING THE AMOUNT OF SUPERINFECTION

A number of factors are known to influence the amount of infection obtained when inoculating a healthy plant with a virus which will cause local lesions. Several of these factors were investigated with the hope that if superinfection of mosaic leaves and the infection of healthy leaves were not similarly influenced by the same factor, information might be gained on the process of superinfection.

Effect of carborundum. The increase in amount of infection resulting from the use of carborundum was determined by inoculating one half of each test leaf without carborundum, then sprinkling it on the opposite half-

leaf and inoculating with the same extract. Mosaic leaves of *N. sylvestris* infected with strain A were inoculated with extracts of strain C diluted 1:250. Healthy leaves of *N. sylvestris* were inoculated with extracts of strain C diluted $1:10^4$. In three trials an average of 12 lesions per half-leaf were produced on mosaic leaves in the absence of carborundum, and 94 per half-leaf inoculated with carborundum. The half-leaf averages for healthy plants were 18 without carborundum, 122 with carborundum. Thus the presence of carborundum increased the number of lesions 7.8 times on mosaic plants and 6.8 times on healthy plants. The abrasive evidently had about the same effect on healthy leaves as on mosaic leaves.

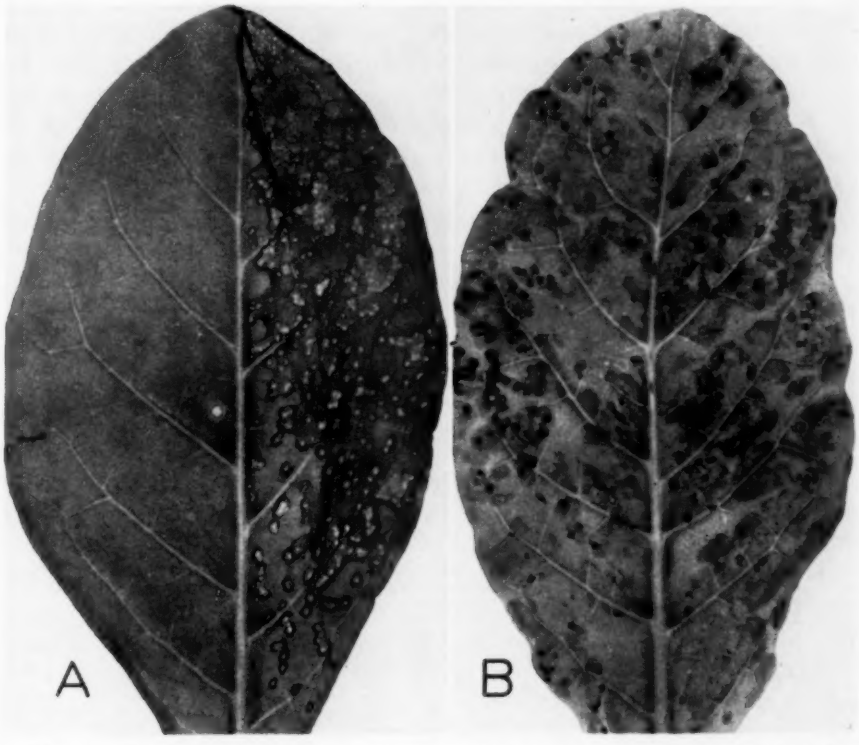

FIG. 3. The local application of tobacco mosaic virus (strain A) resulted, in 4 days, in nearly complete immunity in the left half of a previously healthy leaf of *Nicotiana sylvestris* (A), but no additional resistance in the left half of a mosaic leaf of *N. sylvestris* (B) to inoculation of the entire leaves with a 1:250 dilution of strain C.

Effect of light and darkness. Bawden and Roberts (3) have shown that a period of darkness before inoculation increases the susceptibility of healthy tobacco to a number of viruses. The effect of previous darkness on the amount of superinfection was determined by placing mosaic plants of *N. sylvestris* infected with strain A in complete darkness for 15 hr. At 8 A. M. one half of each leaf was inoculated with strain C diluted 1:250. After 9 hours' exposure to sunlight the opposite half-leaf was inoculated with an aliquot of the same inoculum. Healthy plants used as controls were treated

in the same way, except that they were inoculated with a $1:10^4$ dilution of strain C.

In four trials, an average of 48 lesions per half-leaf was produced on mosaic leaves inoculated immediately after the dark period, while an average of 82 lesions was produced by inoculations made on the opposite half-leaves after a period of sunlight. On the healthy control plants the averages per half-leaf were 65 lesions after the dark period and 138 lesions after the light period. On mosaic plants 1.7 times, and on healthy plants 2.1 times, as many lesions were obtained by inoculations made after the light period as after the dark period. Under the conditions of the experiments, illumination influenced the susceptibility of mosaic plants to superinfection to about the same extent as it influenced the susceptibility of healthy plants.

These results differ from those of Bawden and Roberts (3) in that less infection was secured after the dark period than after the light period. The reason for this difference is not apparent, but may be related to the short period of darkness, or to the length of day prior to the start of the experiments. The present tests were made during the winter when the amount of light was apparently the principal factor limiting the rate of plant growth.

Post-inoculation effect of Phytolacca extract. It has been shown by Stanley (15) and others that certain virus inactivators are effective when applied to leaves within a short time after inoculation. In comparing the response of mosaic and healthy leaves to this type of treatment a clarified extract of leaves of *Phytolacca americana* L. was used as the inactivator. Mosaic leaves of *N. sylvestris* infected with strain A were inoculated with a $1:250$ dilution of strain C, and healthy *N. sylvestris* leaves were inoculated with a $1:10^4$ dilution of strain C. After 40 min. one half of each leaf was wiped with a $1:10$ dilution of the Phytolacca extract.

The application of inhibitor reduced the numbers of lesions on both mosaic and healthy leaves, the average reduction being 86.5 per cent on mosaic leaves and 91.3 per cent on healthy leaves. Apparently there was no difference between mosaic and healthy leaves in the reaction to inhibitor applied immediately after inoculation.

Since all of the treatments had about the same effect on infection of healthy leaves as on superinfection of mosaic leaves, it seemed likely that the fundamental processes leading to the establishment of virus in the tissues may be similar in both cases.

VARIATION IN THE SUPERINFECTING CAPACITIES OF NECROTIC-TYPE STRAINS OF TOBACCO MOSAIC VIRUS

In some of the previously reported experiments strains C and H produced more lesions on mosaic leaves than did the other strains. In order to determine quantitatively any differences between the strains, trials were made in which different strains were inoculated on opposite halves of mosaic leaves. Since there were six strains to be compared, strain C was selected

as a standard and compared with each of the other five strains. Two dilutions of each strain were compared with two dilutions of the standard on the opposite halves of eight leaves on two plants. Preliminary trials showed that in order to get countable numbers of lesions it was necessary to dilute strains C and H 1 : 250 and 1 : 1250 when mosaic leaves were to be inoculated. Strains D, E, F, and G were diluted 1 : 5 and 1 : 25.

The superinfecting capacities of the six necrotic strains were compared on mosaic leaves of *N. sylvestris* and *N. plumbaginifolia* infected with strain A and on the same species infected with strain B. Mosaic leaves that were distorted or did not have approximately the same amount of dark green tissue on both sides of the midrib were not used. The infectivities of the six extracts were compared on healthy plants of these species, using dilutions of $1 : 10^4$ and $1 : 5 \times 10^4$ of each extract. Strain G was not inoculated to *N. plumbaginifolia* since it produced only mosaic on this plant.

The results of these comparisons are in table 2. Superinfection with

TABLE 2.--A comparison of the superinfecting capacities of necrotic-type strains of tobacco mosaic virus on two species of Nicotiana infected with two mosaic-type strains of tobacco mosaic virus

Pairs of superinfecting strains	Lesions per half-leaf (av. of 3 trials) produced on: N. sylvestris infected with:			N. plumbaginifolia infected with:		
	Strain A	Strain B	Healthy	Strain A	Strain B	Healthy
C	115	105	108	30	17	114
D	40	11	24	39	13	105
C	68	87	121	30	8	122
E	30	6	48	51	4	142
C	119	166	116	54	34	57
F	37	20	57	62	11	78
C	132	153	113	...	...	...
G	7	9	16	...	...	...
C	117	165	106	70	48	164
H	79	102	26	1	1	6

Dilutions used to inoculate infected plants were, for strains C and H, 1:250 and 1:1250; for strains D, E, F, and G, 1:5 and 1:25. All strains were inoculated to healthy plants at dilutions of $1:10^4$ and $1:5 \times 10^4$.

strain C occurred much more readily than with strains D, E, F, or G. More lesions were produced by strain C on mosaic leaves of *N. sylvestris* even though the inoculum was 50 times more dilute than the inocula of the other strains. While extracts of strain C were somewhat more infectious than the other extracts when assayed on healthy plants, this difference was much less than that introduced by dilution.

When assayed on healthy *N. plumbaginifolia* the infectivities of strains D, E, and F were approximately the same as infectivity of strain C. On infected plants, however, only slightly greater numbers of lesions were produced by strains D, E, and F at concentrations 50 times greater than the concentration of strain C.

In comparisons on *N. sylvestris* strain H was similar to strain C in that it produced approximately the same numbers of lesions at the same dilution.

N. plumbaginifolia, however, showed considerable resistance to infection by strain H, only few lesions being produced on either healthy or infected plants. Since the symptoms produced on *N. tabacum* by strains C and H were similar, the data are included as evidence that these two strains were not identical. A further difference was that strain H infected tomato, whereas strain C did not.

RATE OF MULTIPLICATION AND SUPERINFECTING CAPACITY OF NECROTIC-TYPE STRAINS OF TOBACCO MOSAIC VIRUS

It might be assumed that strains which superinfect readily are more distantly related to the immunizing strain than are strains which superinfect less readily. However, infection with strain A afforded about the same degree of protection against any one necrotic-type strain as did infection with strain B. Such differences as did appear were minor ones and were not consistent for the same strains on the two hosts. For example, strain C produced slightly more lesions on *N. sylvestris* infected with strain B than on plants infected with strain A. On *N. plumbaginifolia,* however, strain B appeared to give greater protection against strain C than did strain A. It is perhaps incorrect to assume that strains A and B are not closely related because the diseases they produce are dissimilar, but in the absence of any data to the contrary it was thought that greater differences existed between these two strains than between any others that were available. It may be mentioned that in *N. tabacum* the strain B, as was also reported by Holmes (6), did not completely protect against strain A, as judged from the appearance of systemic mosaic symptoms. It appeared that the major differences in the amount of superinfection were due to differences between the necrotic-type strains, rather than to differences between the immunizing strains.

The necrotic-type strains could be placed in two groups, those which superinfected readily (C and H), and those which did so less readily (D, E, F, and G). In an effort to determine the nature of this difference the rate of multiplication of the necrotic-type strains in leaves of *N. sylvestris* was determined. Each of five necrotic-type strains (undiluted extract) was inoculated to one half-leaf of healthy *N. sylvestris,* the opposite half-leaf being inoculated with strain C. Samples were taken at four successive 12-hr. intervals after inoculation by punching five disks 4 mm. in diameter from the inoculated leaves. The leaves became necrotic between 36 and 48 hr. after inoculation, so that the 48-hr. samples consisted entirely of necrotic tissue. The samples were frozen immediately and thawed just before being ground for assay. The final sample was frozen for 12 hr. or more. Extracts were prepared by grinding the five disks in 1 ml. of buffer and diluting this 1 : 10. Each pair of samples taken at one of the four time intervals was inoculated to opposite half-leaves of the *N. tabacum* × *N. glutinosa* hybrid, using one leaf on each of four plants to make a 4 × 4 Latin square arrangement with the four time intervals. The results are in table 3.

The differences between the concentrations of the different strains in *N. sylvestris* 48 hr. after inoculation were consistent and marked. Strains C and H remained at a rather low concentration, while strains D, E, F, and G showed a considerable increase in concentration of active virus. Since the 48-hr. sample in each case consisted entirely of necrotic tissue, it was concluded that the figures for the final samples represent the ultimate extent to which the viruses multiplied before the tissue became necrotic. There were few or no differences observable in the time required for each virus to produce necrosis.

The two strains, C and H, which superinfected readily both caused necrosis of healthy tissue with relatively little increase of virus (Table 3). The four strains D, E, F, and G, which superinfected less readily, multiplied more extensively in healthy tissue before necrosis occurred.

TABLE 3.--Extent of multiplication of necrotic-type strains of tobacco mosaic virus in Nicotiana sylvestris leaves between the time of inoculation with undiluted extracts and complete collapse of the tissue

Interval between inoculation and sampling (hours)	Average[a] lesions per half-leaf of hybrid produced by standard samples of leaves previously inoculated with the following strains:					
	C	D	E	F	G	H
12	9	6	2	5	2	2
24	7	6	3	10	5	1
36	12	66	31	79	41	7
48	25	181	158	174	216	28

[a]Average of 12 half-leaves in three trials, except that for strain C the figures are the average of 60 half-leaves in 15 trials.

A similar series of trials measuring the multiplication of the six strains in *N. plumbaginifolia* gave the same results. Strains C and H multiplied much less extensively than did strains D, E, F, and G. It seems significant that in both hosts the two strains which produce relatively numerous lesions on mosaic plants were able to cause necrosis of healthy tissues without multiplying extensively.

DISCUSSION

The results reported herein differ in some respects from those reported by Kunkel (10), who also used *N. sylvestris* and at least one of the strains (D) used in the present investigation. He reported that the necrotic-type strain produced no local lesions when inoculated to mosaic leaves of *N. sylvestris*. The dilution of extract used for inoculation was not stated, but judging from the numbers of lesions produced on healthy leaves the infectivity was much less than that of the inoculum used in the present study. The fact that carborundum was not used was undoubtedly a contributing factor also. Since the present work showed that with some strains, of which D was one, highly infective inoculum is necessary to produce superinfection, it seemed that the apparent variance from Kunkel's results could

be explained on the basis of differences in the infectivities of the extracts and in the use of carborundum.

As a tentative explanation for the occurrence of superinfection the following hypothesis is proposed. A second strain of virus apparently can be introduced into a cell already containing another strain. Whether or not visible symptoms will result depends upon the amount of subsequent multiplication. In the cells of the dark green areas of mosaic leaves presumably there is available a sufficient amount of necessary constituents, not used by the mosaic-type strain, to allow some multiplication of the introduced necrotic-type strain. If the strain is capable of causing necrosis with relatively little multiplication, then many local lesions will be produced. There are evidently relatively few areas of dark green tissue which can support sufficient multiplication to allow the production of necrotic lesions by strains which must multiply rather extensively to do so.

Evidence in support of this hypothesis might have been obtained by demonstrating that strains D, E, F, and G multiplied in the dark green areas of mosaic leaves without causing necrosis. A few attempts were made to determine the relative amounts of two strains present in necrotic tissue of superinfected mosaic plants. It was found that the mosaic-type strain was present greatly in excess of the necrotic-type strain. It did not appear to be practical, with the methods available, to attempt to assay for the necrotic-type strains in the presence of such an excess of the mosaic-type strain.

The difference in virus concentration between the light and dark green areas of mosaic leaves was striking and consistent, regardless of the age of the leaf sampled. A problem which seems worthy of further investigation is the mechanism which restrains the mosaic-type virus in the dark green areas, yet allows a certain amount of multiplication by an introduced necrotic-type strain. The absence of inclusion bodies in the dark green tissue, as reported by Hoggan (4), is also indicative of some factor restraining maximum development of the virus.

The numerous reports on the use of the cross-protection test in identifying various viruses indicates that superinfection does not occur commonly. It may be that most viruses are not present in plant extracts in sufficient concentration to cause superinfection, or that other viruses are unable to multiply sufficiently in any tissue of the test plant to produce visible symptoms. If superinfection occurs without production of additional symptoms, it might be more easily detected with some of the less stable viruses than with tobacco mosaic virus.

It is apparent from the results presented that, in some cases at least, "acquired immunity" is relative rather than complete. It seems incorrect to state categorically that only one strain of a virus may multiply in a single cell. The development of mutant strains of virus may be supposed to depend upon the multiplication of the mutant in a cell containing at least a small quantity of the parent virus. The rather common occurrence of mixtures of strains of tobacco mosaic virus in naturally infected plants is probably due to the inability of one strain to completely suppress the other.

SUMMARY

When mosaic leaves of *Nicotiana sylvestris* infected with tobacco mosaic virus were inoculated with strains of the virus causing local lesions on this host, lesions were produced on the mosaic leaves. Such lesions were much fewer in number than on healthy leaves, were slower to appear, and remained smaller. This superinfection occurred with each of six necrotic-type strains of tobacco mosaic virus when inoculated to mosaic leaves of *N. sylvestris* infected with any one of 18 mosaic-type strains or isolates.

The lesions produced on mosaic leaves appeared only in the dark green tissue. This tissue contained much less of the mosaic-type strain than did the light green areas. No lesions were produced on halves of *N. sylvestris* leaves which had been inoculated 4 days previously with mosaic-type strains. The concentration of virus in these half-leaves was greater than in the dark green areas of mosaic leaves. Superinfection thus did not occur in tissues with a high virus content.

The inoculation of mosaic leaves with mosaic strains of tobacco mosaic virus did not increase resistance to superinfection by necrotic-type strains. From this it was concluded that there were no virus-free cells in the mosaic leaves, and that the lesions resulted from the multiplication of the necrotic-type strain in cells already containing a mosaic-type strain.

The use of carborundum, variations in illumination, and the application of virus inactivator influenced the amount of superinfection of mosaic leaves to about the same extent as they influenced infection of healthy leaves.

Two of the six necrotic-type strains produced greater numbers of lesions on mosaic leaves than did the other four strains. When inoculated to healthy leaves of *N. sylvestris* these two strains caused complete necrosis with markedly less multiplication than did the other four strains.

Apparently, in the dark green areas of mosaic leaves the necessary constituents unused by the mosaic-type strain are sufficient to allow some multiplication of an introduced strain. If these introduced strains produce necrosis without extensive multiplication, many local lesions are produced.

DIVISION OF TOBACCO, MEDICINAL, AND SPECIAL CROPS
U. S. DEPARTMENT OF AGRICULTURE
AND
WISCONSIN AGRICULTURAL EXPERIMENT STATION
MADISON, WISCONSIN

LITERATURE CITED

1. BALD, J. G. Potato virus X: effectiveness of acquired immunity in older and younger leaves. Austral. Council Sci. and Indus. Res. Jour. **21**: 247–251. 1948.
2. BAWDEN, F. C. Studies on a virus causing foliar necrosis of the potato. Proc. Roy. Soc. London (B) **116**: 375–395. 1934.
3. ———, and F. M. ROBERTS. Photosynthesis and predisposition of plants to infection with certain viruses. Ann. Appl. Biol. **35**: 418–428. 1948.
4. HOGGAN, ISME A. Cytological studies on virus diseases of Solanaceous plants. Jour. Agr. Res. [U.S.] **35**: 651–671. 1927.
5. HOLMES, FRANCIS O. Accuracy in quantitative work with tobacco mosaic virus. Bot. Gaz. **86**: 66–81. 1928.
6. ———. A masked strain of tobacco-mosaic virus. Phytopath. **24**: 845–873. 1934.

7. JOHNSON, E. M., and W. D. VALLEAU. Susceptibility of tobacco plants visibly affected with mild tobacco mosaic to other strains of the virus. Ky. Agr. Exp. Sta. Res. Bul. 360: 192–201. 1935.
8. JOHNSON, JAMES. Virus attenuation and the separation of strains by specific hosts. Phytopath. **37**: 822–837. 1947.
9. KÖHLER, E., and IRMGARD HAUSCHILD. Betrachtungen und Versuche zum Problem der "erwarbenen Imunität" gegen Virusinfektionen bei Pflanzen. Zuchter **17/18**: 97–105. 1947.
10. KUNKEL, L. O. Studies on acquired immunity with tobacco and aucuba mosaics. Phytopath. **24**: 437–466. 1934.
11. MATTHEWS, R. E. F. Criteria of relationship between plant virus strains. Nature **163**: 175. 1949.
12. MCKINNEY, H. H. Virus-antagonism tests and their limitations for establishing relationships between mutants, and non-relationships between distinct viruses. Amer. Jour. Bot. **28**: 770–778. 1941.
13. SALAMAN, REDCLIFFE N. Protective inoculation against a plant virus. Nature **131**: 468. 1933.
14. SILBERSCHMIDT, KARL. The effect of "premunity" on the type of lesions induced by aucuba mosaic virus. Tijdschr. Pl.-ziekten. **55**: 166–178. 1949.
15. STANLEY, W. M. Chemical studies on the virus of tobacco mosaic. I. Some effects of trypsin. Phytopath. **24**: 1055–1085. 1934.
16. THUNG, T. H. Smetstof en plantencel bij enkele virusziekten van de tabaksplant. Z. Ned. Indisch Natuurwetensch. Congr. Bandoeng, Java. 450–463. 1931.

INHERITANCE OF A NECROTIC–LESION REACTION TO A MILD STRAIN OF TOBACCO MOSAIC VIRUS[1]

PAUL V. V. WEBER

(Accepted for publication February 3, 1951)

The production of necrotic local lesions by certain *Nicotiana* hosts in response to inoculation with the tobacco mosaic virus has gained wide usage in virus research. The reaction is also recognized as being of paramount importance in the development of mosaic-resistant commercial varieties of tobacco.

Although previous investigators have demonstrated the typical genetic behavior of the necrotic local-lesion reaction in *Nicotiana*, they have generally used ordinary severe strain, or mixed strains, of the virus. It is now known that some strains of this virus yield opposite reactions to that of the severe strain on certain species of *Nicotiana*. Consequently it seemed desirable to investigate and compare the inheritance of the two types of reactions.

The inheritance of the necrotic local-lesion reaction induced on a number of *Nicotiana* species by an ordinary severe strain of the tobacco mosaic virus was first studied by Holmes (3) in 1934. Included in his list of hosts yielding this response were *N. langsdorffii*, a necrotic variety of *N. sanderae*, *N. acuminata*, *N. rustica*, and *N. glutinosa*. The reactions of *N. langsdorffii* and the *N. sanderae* variety were found to be inherited as single dominant factors. The segregation obtained with *N. acuminata*, however, was in a ratio of 5.5 necrotic : 1 mottling, rather than the expected 3 : 1 ratio.

In later articles, Holmes (4, 6) showed that the necrotic reactions of *N. rustica* and *N. glutinosa* to the virus were usually inherited as single dominant factors. He designated the genes which determine the necrotic reactions of these hosts *NN*, and the corresponding recessive genes which determine the chlorotic or systemic-mottling reactions typical of most other *Nicotiana* species, *nn*. Subsequent work by Kostoff and Georgieva (11) seems to verify these findings.

Holmes (5) demonstrated also that one of two necrotic local-lesion responses found in pepper (*Capsicum* spp.) to the tobacco mosaic virus was inherited as a single dominant character, whereas the other was incompletely dominant. Earlier (3) he had described a necrotic-lesion reaction to this virus in eggplant (*Solanum* spp.) which appeared to be inherited as an incompletely dominant character.

Kunkel (12) first reported a type of necrotic local-lesion reaction nearly

[1] Aided by a grant from the Wisconsin Alumni Research Foundation.

The author is indebted to Dr. James Johnson for advice and criticism during the work and preparation of the manuscript and for generous provision of material.

Illustrations were prepared by the University of Wisconsin Photographic Laboratory, or by Eugene H. Herrling.

identical to that studied in the present investigation. In 1932 he found that the aucuba ("yellow") tobacco mosaic strain yielded necrotic local lesions not only on *N. langsdorffii*, *N. acuminata*, *N. rustica*, and *N. glutinosa*, but also on *N. sylvestris*, *N. tabacum* var. Burley, *N. tabacum* var. Little Orinoco, and others. Later, Kunkel (13) showed that, whereas aucuba mosaic produced necrotic local lesions on *N. sylvestris*, an "ordinary" strain of the tobacco mosaic virus produced only the usual systemic mosaic.

Valleau (15) also found a necrotic local-lesion reaction in certain ordinary varieties of tobacco (*N. tabacum*). He used the "white" mosaic and the aucuba mosaic strains of the tobacco mosaic virus. He concluded that a necrotic spotting or sensitivity factor, as found in a line of *N. tabacum* var. Judy's Pride (a White Burley type), was controlled by a single dominant pair of genes. The possibility that this type of reaction might also be inherited as incompletely dominant genes was suggested by Valleau on the basis of results he obtained with *N. tabacum* var. Ambalema. The genes determining these necrotic spotting reactions were designated N'N' by Valleau and Johnson (17). Several varieties of burley and dark tobacco grown in Kentucky were listed as carrying this gene pair.

The chief purpose of the present investigation was to determine the mode of inheritance of the necrotic local-lesion reaction of certain *Nicotiana* species to an atypical strain (9) of the tobacco mosaic virus, referred to as the "mild" strain, and to compare this with results of others who used "ordinary" or "severe" strains of the virus.

MATERIALS AND METHODS

Four suitably reacting hosts were available for use as necrotic-type parents at the initiation of this study. These were *N. sylvestris*, *N. longiflora*, Clayton's TL106 (1), and *N. tabacum* var. Daruma.

Johnson (9) found that each of these yielded typical necrotic local lesions when inoculated with a mild green strain (attenuated) of the tobacco mosaic virus, but that they failed to produce necrotic local lesions in reaction to an ordinary, severe strain of this virus. Only symptoms of the typical systemic mosaic disease resulted in this latter case. The reactions of *N. sylvestris* of these two strains (Fig. 1) are typical of this group.

Clayton's TL106 is a wildfire-resistant type of tobacco selected by Clayton (1) from the cross *N. tabacum* × *N. longiflora*.

Although *N. tabacum* var. Daruma appears to be a Japanese variety, its exact parentage was not known.

In addition, Holmes Samsoun and Kentucky 56, types of tobacco which react with necrotic local lesions to both strains of the virus, were used as parent plants in two of the crosses. These parents carry the *NN* genes derived from *N. glutinosa*.

Two main classes involving six lines of crosses were studied during this investigation. These were as follows:

A. Interspecific crosses.
   1. *N. tabacum* var. Havana Seed (systemic mottling) × *N. sylvestris* (necrotic lesions).[2]
   2. *N. longiflora* (necrotic lesions) × *N. alata* (systemic mottling).

B. Intervarietal crosses.
   1. *N. tabacum* var. Havana Seed (systemic mottling) × Clayton's TL106 (necrotic lesions).

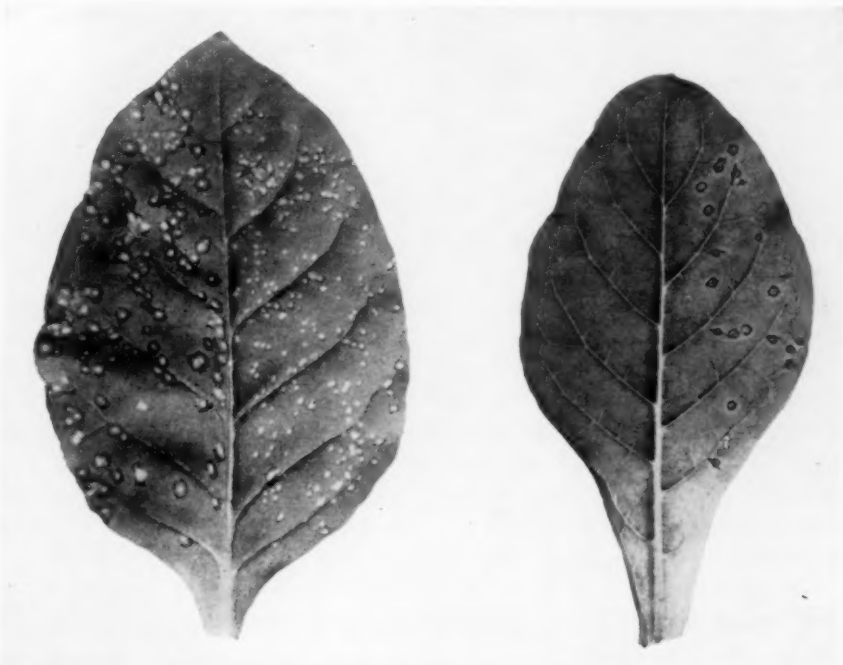

FIG. 1. Leaves of the tobacco hybrid *Nicotiana tabacum* × *N. glutinosa*, left, and of *N. sylvestris*, right. The left side of each leaf shown was wiped with a dilution of the severe strain of the tobacco mosaic virus and the right side with the mild strain. Note that only the mild strain of the virus produces necrotic local lesions on *N. sylvestris*.

   2. *N. tabacum* var. Havana Seed (systemic mottling) × *N. tabacum* var. Daruma (necrotic lesions) and *N. tabacum* var. "American"[3] (systemic mottling) × Daruma.
   3. Holmes Samsoun (*NN*) × *N. tabacum* var. Daruma (necrotic lesions).
   4. Kentucky 56 (*NN*) × Havana Seed (systemic mottling).

The systemic mottling varieties used in these crosses showed typical systemic symptoms of tobacco mosaic, corresponding in severity to the strain of the virus used.

[2] The female parent will always be written first in the cross designations used in this paper.

[3] "American" is not a United States type but evidently is of South American origin.

For virus material, two single-lesion, pure-line strains (9) of ordinary tobacco mosaic virus (*tobacco virus 1*) were used throughout this investigation. Although it is recognized that several other similar strains exist, these strains will be referred to simply as the "mild" and "severe" strains for purposes of this study.

The "mild," or attenuated, green mosaic strain was originally isolated by Johnson (9) from noninoculated leaves of sea-holly (Buttonsnakeroot), *Eryngium equaticum* L. The chief characteristic of this strain from the standpoint of this investigation is that it produces typical necrotic local lesions on *N. sylvestris* as well as on *N. glutinosa* and the hybrid host (*N. tabacum* × *N. glutinosa*) (Fig. 1). Strains similar to, or identical with, the mild strain have been isolated several times from collections of tobacco mosaic from various Wisconsin tobacco farms. Hence, the strain is probably commonly present in ordinary field tobacco mosaic virus.

The "severe" green mosaic strain of the tobacco mosaic virus was originally isolated from a field collection of mosaic tobacco. The chief characteristic of this strain is that it does not produce necrotic local lesions on *N. sylvestris* (Fig. 1) and similarly reacting hosts, but systemic mosaic instead. As far as tested, the severe strain has induced much the same reactions in *Nicotiana* species as those reported by Holmes (7) for the typical distorting strain of the tobacco mosaic virus.

A type of aucuba mosaic, namely, a mild-yellow mosaic strain, was included in occasional trials for comparative purposes. This strain resembles the mild (attenuated) green mosaic strain in that it produces necrotic local lesions on *N. sylvestris* and on several other *Nicotiana* hosts which yield only systemic mottling with the severe strain. The mild-yellow strain is clearly distinguished from the mild-green mosaic strain on the basis of symptoms on Havana Seed.

Extracts for inoculations were obtained by expressing juice from diseased tobacco plants. These were stored as stock inoculum under toluene at 4° C. to reduce fermentation.

The plants to be inoculated were grown in composted soil in 4-in. pots. Greenhouse temperature normally ranged from 75° to 85° F. The plants were usually grown until the largest leaves were from 3 to 5 in. long before they were inoculated. It was desirable to use only stocky, green, vigorously growing plants in order to obtain typical necrotic local lesions.

Inoculations were made by dipping a cheesecloth pad in the properly diluted infective extract and lightly wiping it over the surface of the leaves previously dusted with carborundum powder. Usually at least three leaves of each plant were inoculated. For the comparison of two or more strains of the virus, inoculations were often made on opposite half-leaves of the same plant.

The *N. tabacum* × *N. glutinosa* hybrid (8) and either Daruma or *N. sylvestris* were included in each trial as control plants. These indicated the concentration of the inoculum, and the latter two hosts, in addition, checked

the absence of the mild strain in the severe strain inoculum. *N. acuminata*, which shows necrotic local lesions with the severe strain and only systemic mottling with the mild strain, was occasionally included in the tests to check the absence of the severe strain in the mild strain inoculum.

A plant was not considered to be of the necrotic type unless at least five necrotic local lesions developed on at least one inoculated half-leaf. This procedure allowed for such occasional contamination of strains as might

TABLE 1.--The reaction of certain Nicotiana species and varieties when inoculated on opposite half-leaves with the mild and severe strains of tobacco mosaic virus

Species or variety	Number of plants inoculated	Reaction of plants		
		Necrotic with both strains	Necrotic with mild strain only	Mottling with both strains
		(Number)	(Number)	(Number)
N. sylvestris	25	0	25	0
N. longiflora	10	0	10	0
N. alata	30	10	2	18
N. tomentosa	8	0	0	8
N. tomentosiformis	7	0	0	7
N. glutinosa	4	4	0	0
Holmes Samsoun	61	61	0	0
Clayton's TL106	9	0	9	0
N. tabacum var.				
atropurpurea	14	0	0	14
Connecticut Broadleaf	7	0	7	0
Cuban	11	0	11	0
Havana Seed	40	0	0	40
Maryland Broadleaf	11	0	11	0
Pennsylvania Broadleaf	14	0	14	0
White Burley				
Judy's Pride[a]	3	0	0	3
Kelley	7	0	7	0
White Stem Orinoco	24	0	24	0
Wisconsin Seedleaf	14	0	0	14
Yellow Pryor	7	0	7	0
Ambalema[b]	7	0	0	7
"American"[c]	29	0	0	29
Daruma	25	0	25	0
Bonanza	11	0	10	1
Sumatra	9	0	8	1
Turkish				
Xanthia	11	0	11	0
Three unnamed varieties	19	0	19	0
Four named varieties	75	0	35	40
Five unnamed varieties	54	0	0	54

[a] Twenty additional plants, inoculated only with the mild strain, all yielded mottling symptoms and no necrotic local lesions.

[b] The mottling symptoms are very mild or not visible, but the virus is recoverable from noninoculated leaves.

[c] "American" is a foreign variety.

result from applying two virus strains on opposite half-leaves. Usually, from 25 to 100 such lesions developed. Failure to develop necrotic local lesions was not recorded until at least 3 days after inoculation, and usually not until 4 to 7 days had elapsed. It was assumed that a plant failing to develop necrotic local lesions in this period would finally become systemically infected.

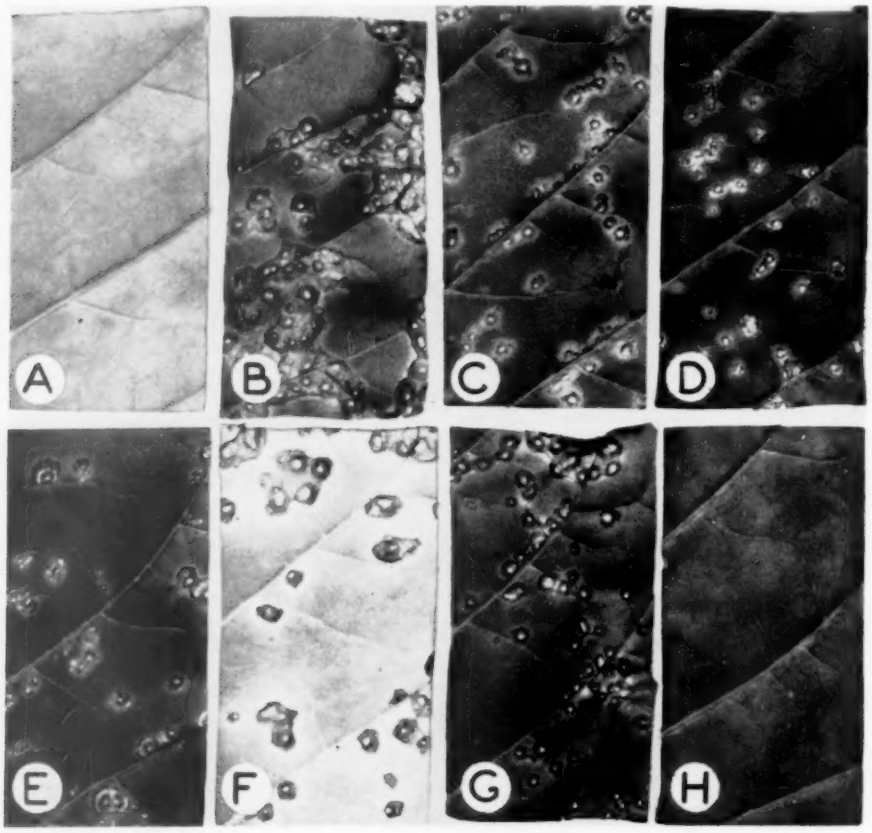

FIG. 2. Portions of leaves of eight varieties of *N. tabacum* showing reactions to the mild strain of the tobacco mosaic virus. A. Havana Seed. B. Cuban. C. White Stem Orinoco. D. Yellow Pryor. E. Connecticut Broadleaf. F. Kelley White Burley. G. Maryland Broadleaf. H. *N. tabacum* var. *atropurpurea*. Photographed by Eugene H. Herrling.

In the interpretation of experimental results, observed ratios were tested for goodness of fit as outlined by Sinnott and Dunn (14).

REACTIONS OF CERTAIN NICOTIANA SPECIES AND VARIETIES

Although the reactions of some species of *Nicotiana* and of some varieties of *N. tabacum* to the mild and severe strains of tobacco mosaic were known, it seemed desirable at the outset, and during the course of this investigation, to verify or determine the reactions of all available host plants.

The results of a group of such tests are in table 1. It is obvious from this test that a few *Nicotiana* species, and several varieties of *N. tabacum*, yield local lesions with the mild strain only.

In the course of this investigation it was found that several of the standard, commercial varieties of tobacco grown in the United States, while becoming systemically infected with the severe strain of the tobacco mosaic virus, are evidently resistant to systemic infection by the mild strain by virtue of their necrotic-lesion reaction (Fig. 2).

INHERITANCE IN INTERSPECIFIC CROSSES

N. tabacum (*systemic mottling*) × *N. sylvestris* (*necrotic lesions*). The F_1 generation, but no F_2 seed, was obtained from this cross. Sterility in this hybrid has been encountered by others. All of 100 F_1 plants tested from this cross yielded a peculiar type of necrotic local-lesion reaction to the mild strain. This reaction appeared as poorly demarcated necrotic local lesions that were much smaller and less regularly shaped than those typical of the necrotic-type parent, *N. sylvestris*. In most instances, these lesions appeared to be intermediate in size and type between a typical necrotic local lesion and no necrotic reaction to inoculation. The typical intermediate-type reactions usually occurred on the middle leaves of the plant. The severe strain induced systemic mottling but no necrotic lesions in these plants.

In addition, the mild strain, like the severe strain, became systemic in the F_1 plants and could be recovered from the new noninoculated leaves. In this respect, the F_1 behaved similarly to its systemic-mottling parent, *N. tabacum*.

From these results, it is concluded that the necrotic-lesion reaction of *N. sylvestris* is incompletely dominant in the F_1 of the cross with Havana Seed.

N. longiflora (*necrotic lesions*) × *N. alata* (*systemic mottling*). With this cross, as with the previous one, the F_1 generation, but no F_2 was obtained. The 24 F_1 plants grown all yielded necrotic local lesions with the mild strain, as did the *N. longiflora* parent. Eighteen F_1 plants inoculated with the severe strain yielded only systemic mottling. Many repetitions of a backcross, with *N. alata* as the pollen parent, resulted in sufficient backcross seed for further study. When the mild strain was inoculated to 194 plants of this backcross generation, 97 plants yielded typical necrotic local lesions, and 97 plants failed to react necrotically. Hence a segregation in an exact 1 : 1 ratio was obtained. In no case was the mild strain observed to produce a systemic mottling in the new noninoculated leaves of a necrotic-type plant. One hundred and thirteen of these same plants had been inoculated at the same time, on the opposite half of the leaves, with the severe strain of the virus. All these latter inoculations resulted in systemic mottling only.

It is concluded from the results of this cross that the necrotic local-lesion

reaction of *N. longiflora* to the mild strain is determined by a single Mendelian factor which is dominant in crosses with the systemic-mottling variety of *N. alata*.

INHERITANCE IN INTERVARIETAL CROSSES

N. tabacum var. Havana Seed (systemic mottling) × Clayton's TL106 (necrotic lesions). Efforts to obtain viable seed from the cross *N. tabacum* × *N. longiflora* were unsuccessful. E. E. Clayton (1), however, secured a fertile line which he designated TL106. Since this host had been found in this laboratory (9) to react uniformly with necrotic local lesions to inoculations with the mild strain, TL106 was substituted for *N. longiflora* in a cross with Havana Seed.

The resulting segregations are in table 2. Again the intermediate type

TABLE 2.--The reaction of the F_1, F_2, and F_3 generations of the cross N. tabacum var. Havana Seed (systemic mottling) × Clayton's TL106 (necrotic lesions) when inoculated with the mild and the severe strains of the tobacco mosaic virus

Generation	Plants inoculated with mild strain	Reaction of plants to the mild strain			χ^2 for 1:2:1 ratio	Plants inoculated with severe strain[a]
		Necrotic	Intermediate	Mottling		
	Number	Number	Number	Number		Number
F_1 and reciprocal	78	0	78	0		78
F_2 and reciprocal	754	192	358	204	2.30[b]	45
Two F_3 families	72	72	0	0		22
Eight F_3 families	329	79	150	100	5.24[c]	18
One F_3 family	50	0	0	50		22

[a]All plants of all generations that were inoculated with the severe strain yielded only systemic mottling to that strain.

[b]P = 0.32--a fair fit since χ^2 is well below 5.99.

[c]P = 0.08--a rather poor fit since χ^2 is not far below 5.99.

of reaction, as seen in the cross with *N. sylvestris*, was found. Following inoculations with the mild strain, the F_2 generation segregated in fairly close agreement with a 1:2:1 ratio of plants showing typical necrotic lesions : plants yielding intermediate-type lesions : plants showing systemic mottling, respectively. Leaves illustrating the three reaction classes obtained in the F_2 are shown in figure 3.

The F_3 generation substantiated the hypothesis that a single pair of incompletely dominant factors determined the necrotic reaction of Clayton's TL106, although the segregation of reactions of the limited number of heterozygous F_3 families did not approximate a 1:2:1 ratio so closely as did the F_2 generation. However, the presence of the expected classes of F_3 families in numbers suggesting a 1:2:1 ratio supports the hypothesis of incomplete dominance.

It is concluded that the necrotic local-lesion reaction of Clayton's TL106 to the mild, or attenuated, green mosaic strain of the tobacco mosaic virus is governed by a single, incompletely dominant factor in crosses with Havana Seed.

For comparative purposes, opposite half-leaf inoculations were made with the mild or attenuated green mosaic strain and a mild-yellow mosaic strain of the tobacco mosaic virus. Nineteen plants of Clayton's TL106, six F_1 plants of the cross TL106 × Havana Seed, and 27 F_2 plants from this cross were tested. The results obtained with these two strains were identical, except for small differences in the type of lesions produced and the greater tendency for the mild-yellow strain to become systemic in TL106. In both cases an F_2 segregation of 7 plants with necrotic lesions : 14 plants

FIG. 3. Reactions typical of the three classes of segregates in the F_2 of the cross Clayton's TL106 (necrotic lesions) × *N. tabacum* var. Havana Seed (systemic mottling). The left side of each leaf shown was wiped with a dilution of the severe strain of the tobacco mosaic virus and the right side with the mild strain. Note the intermediate type of reaction to the mild strain shown by the center leaf.

with intermediate lesions : 6 plants with systemic mottling was obtained. Each plant reacted with either local lesions to both strains or systemic mottling with both strains. This segregation obviously fits a 1 : 2 : 1 ratio. Thus it seems that the necrotic local-lesion reaction of Clayton's TL106 to the mild-yellow mosaic strain is inherited in the same manner as is its reaction to the mild or attenuated green mosaic strain of the virus principally used in this study.

TL106 resembles its *N. tabacum* parent, White Stem Orinoco, more closely than its *N. longiflora* parent both in its habit of growth and the appearance of its necrotic local lesions in reaction to the mild strain. Thus, it seems more likely that the necrotic lesion reaction of Clayton's TL106 was contributed by the *N. tabacum* parent rather than by *N. longiflora*.

N. tabacum (*systemic mottling*) × *Daruma* (*necrotic lesions*). Two

crosses involving the necrotic-lesion reaction of the variety Daruma and the systemic-mottling reaction of Havana Seed types were available for study early in this investigation. These were Havana Seed × Daruma, and the reciprocal, and Wisconsin Seedleaf (systemic mottling) × Daruma. At the same time, a third cross, "American" (systemic mottling) × Daruma, was studied. The results from these three crosses will be considered together.

The combined results, tabulated on the basis of complete dominance of the necrotic-lesion character of Daruma, are in table 3. The fit to a 3:1 ratio of the segregation of necrotic to non-necrotic-type plants is poor in the total F_2, although satisfactory in each of the included F_2 generations. The

TABLE 3.-- The reactions of the F_1, F_2, and F_3 generations of the crosses N. tabacum var. Havana Seed (systemic mottling) × N. tabacum var. Daruma (necrotic lesions) and N. tabacum var. "American" (systemic mottling) × Daruma when inoculated with the mild and the severe strains of the tobacco mosaic virus

Generations and crosses	Plants inoculated with mild strain	Reaction of plants to the mild strain		Dev./S.E. for fit to 3:1 ratio[a]	Plants inoculated with severe strain[b]
		Necrotic	Mottling		
	Number	Number	Number		Number
F_1 (3 crosses)	85	85	0		51
F_2 Havana Seed × Daruma and reciprocal	205	143	62	1.7	175
F_2 Wisconsin Seedleaf × Daruma....	303	212	91	2.0	182
F_2 "American" × Daruma	322	237	85	0.4	197
Total F_2 (3 crosses).	830	592	238	2.5	554
F_3--5 families	262	262	0		
13 families	544	411	133	0.5	
10 families	424	0	424		0

[a] If Dev./S.E. is 2.0 or less, population is probably an example of a 3:1 ratio.

[b] All plants of all generations that were inoculated with the severe strain yielded only systemic mottling to that strain.

segregations of the segregating F_3 families closely fit a 3:1 ratio. The F_3 families were not chosen at random (although F_2 parental reactions were not exactly known), and therefore their segregations with respect to each other are not expected to conform to any genetic ratio.

A trial was made in which F_1 plants of these three crosses were inoculated with the mild strain only. It was observed, especially in the F_1 generations of the crosses involving Havana Seed types of tobacco, that the necrotic local lesions produced were intermediate in type. Furthermore, a mild systemic mottle or mosaic and sometimes a systemic necrotic symptom developed in the new noninoculated leaves, and the mild strain was recovered from these leaves. Consequently it seemed most likely that the necrotic-

lesion character of Daruma was incompletely dominant in all these crosses, rather than completely dominant as first supposed. Data assembled on the hypothesis of incomplete dominance are in table 4. The F_2 data closely fit the hypothesis in only one case because of the fact that the intermediate class was not clearly recognized until toward the termination of the observations. The segregations of the heterozygous F_3 families, on which observations were made later, correspond more closely to a 1:2:1 ratio. It is concluded from these crosses that the necrotic-lesion character of Daruma is inherited as a single incompletely dominant character.

The F_1 generations of crosses between three commercial varieties of *N. tabacum*, which yield necrotic local lesions to the mild strain, and the systemically mottling Havana Seed variety were also studied. These crosses

TABLE 4.--The incompletely dominant local-lesion character as shown in the crosses N. tabacum var. Havana Seed (systemic mottling) × N. tabacum var. Daruma (necrotic lesions) and N. tabacum var. "American" (systemic mottling) × Daruma when inoculated with the mild strain of the tobacco mosaic virus

Generation and crosses	Plants inoculated	Reaction of plants to the mild strain			χ^2 for fit to 1:2:1 ratio[a]
		Necrotic	Intermediate	Mottling	
	Number	Number	Number	Number	
F_1 (3 crosses)	94	0	94	0	
F_2 Daruma × Havana Seed and reciprocal	96	25	46	25	0.17
F_2 Wisconsin Seedleaf × Daruma	121	41	46	34	7.76
F_2 "American" × Daruma	125	42	48	35	7.51
Total F_2 (3 crosses)	342	108	140	94	12.39
F_3 heterozygous families (ten families)	283	58	146	79	3.40

[a] If χ^2 value is 5.99 or less, population is probably an example of a 1:2:1 ratio.

were: Orinoco × Havana Seed, Havana Seed × Cuban, and Havana Seed × Maryland Broadleaf. Limited trials showed that the mild strain first induced intermediate-type lesions in the F_1 plants, and later systemically infected these plants in each of these crosses, with resulting mottle in the new leaves. These results were consequently similar to those secured with Daruma.

INHERITANCE IN CROSSES WITH THE NN FACTOR

Holmes Samsoun (NN) × N. tabacum var. Daruma (necrotic lesions). In order to determine the relation between the *NN* genes from *N. glutinosa*, and the factors from *N. tabacum* var. Daruma which yield necrotic local lesions with the mild strain but not with the severe, a cross between Holmes Samsoun (*NN*) and Daruma was studied. Holmes Samsoun is a Samsoun-

like line of the necrotic-type tobacco which carries the necrotic factor derived from *N. glutinosa*.

As shown in table 5, the F_1 progeny from this cross did not yield a uniform reaction such as might be expected in an F_1 generation. This was equally true of each of the three repetitions of this cross, the F_1 results of which were combined to make up these data. No certain explanation for these results has been found. Neither contamination of the cross at the time it was made, nor heterozygosity of parental material seems to be a likely explanation. Nor do chromosomal irregularities explain these results. That a nonuniform F_1 is not always to be expected in a cross involving the *NN* factors from *N. glutinosa* and the necrotic-lesion factors from Daruma is shown by results of trials with the F_1 of the cross Daruma and Kentucky 56 (*NN*). Twenty-eight inoculated F_1 plants from this cross uniformly

TABLE 5.--The segregation of the F_1, F_2, and F_3 of the cross Holmes Samsoun (*NN*) and Daruma (necrotic lesions) when inoculated on opposite half-leaves with the mild and the severe strains of tobacco mosaic virus

Generation	Plants inoculated	Reaction of plants: Necrotic with both strains	Necrotic with mild strain only	Mottling with both strains	$\frac{\text{Dev.}}{\text{S.E.}}$ for fit to 3:1 ratio[a]
	Number	Number	Number	Number	
F_1 (3 lines)	215	162	42	11	
F_2, line 2	84	64	0	20	0.3
F_2, line 3	347	261	86	0	0.09
F_3 family 1[b]	57	57	0	0	
F_3 families 2,3,4,5	152	109	43	0	0.9
F_2 families 6,7,8 ...	131	0	131	0	
F_3 family 9	61	23	38	0	

[a] If Dev./S.E. is 2.0 or less, population is probably an example of a 3:1 ratio.

[b] All F_3 families were grown from F_2, line 3.

yielded necrotic local lesions with both strains of the virus. Furthermore, there is no previous report known to the author of a cross involving *N. glutinosa* (*NN*) or its derivatives and *N. tabacum* which has resulted in other than a uniform F_1 generation of necrotic-type plants.

For the purpose of this investigation it is assumed that, although the cause of the aberrant F_1 results is unknown, the subsequent F_2 and F_3 results were not further altered by the same unknown cause.

Three plants from one of the three previously mentioned F_1 progenies between Holmes Samsoun and Daruma were grown and self-pollinated for the production of F_2 seed. The first F_2 line yielded only systemic mottling with both strains of the virus. The second F_2 line (Table 5) segregated in close approximation to a 3:1 ratio of plants yielding necrotic lesions with both strains of the virus to plants yielding systemic mottling with both strains.

The third F_2 line (Table 5) segregated in an almost perfect 3:1 ratio of *NN*-type reactions to Daruma-type reactions. Figure 4 shows reactions typical of the two classes of segregates obtained in this F_2 line. With the exception of one family, the F_3 generation from line 3 segregated according to the single-factor hypothesis. The aberrant segregation of F_3 family 9 may have been due to irregular chromosome behavior.

The conclusion drawn from the results of the crosses between Holmes Samsoun and Daruma is that the well-known necrotic local-lesion factor of *N. glutinosa* is probably a single Mendelian dominant allele of the necrotic-lesion factor of Daruma.

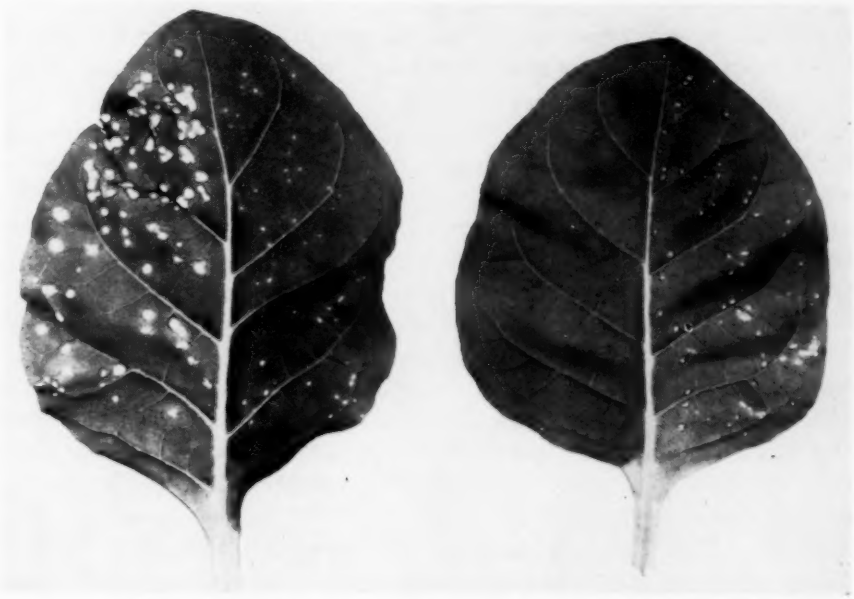

FIG. 4. Reactions typical of the two classes of segregates obtained from the third F_2 line of the cross Holmes Samsoun (*NN*) × *N. tabacum* var. Daruma (necrotic lesions). The left side of each leaf shown was wiped with a dilution of the severe strain of the tobacco mosaic virus and the right side with the mild strain.

Is the necrotic-lesion reaction of Holmes Samsoun to the mild strain determined by the same genes that govern its reaction to the severe strain? The segregation of the second F_2 line (line 2, Table 5) of the cross Holmes Samsoun × Daruma bears on this question. Sixty-four plants in this F_2 family yielded necrotic local lesions with both strains of the virus, whereas 20 plants reacted with systemic mottling to both strains. This segregation is a close fit to a 3:1 ratio. This indicates that the necrotic reactions of Holmes Samsoun (*NN*) to the two virus strains are determined by the same genes. It also shows that the *glutinosa* type of necrotic local-lesion reaction is a single Mendelian dominant allele of the completely systemic mottling reaction of Havana Seed.

Kentucky 56 (*NN*) × *Havana Seed* (*systemic mottling*). The F_2 generation of this cross segregated into 100 plants yielding necrotic local lesions to both the mild and the severe strains of the virus and 40 plants producing only systemic mottling with both strains. This segregation is clearly an example of a 3:1 ratio. These results support the hypothesis that the necrotic local-lesion reactions of Holmes Samsoun to the mild and the severe strains of the tobacco mosaic virus are determined by the same gene pair, namely the *NN* genes. Furthermore, these results again show these *NN* genes to be the dominant alleles of the genes determining the completely systemic mottling reaction of Havana Seed.

DISCUSSION

Holmes (3, 4, 6) has shown that the necrotic local-lesion reaction of certain *Nicotiana* hosts to a distorting, or severe, strain of the tobacco mosaic virus was usually inherited as a single dominant character. In contrast to this, the necrotic reaction to the mild strain of the virus, studied in the present investigation, was inherited in one of two ways, depending on the parent hosts used.

When Kentucky 56, which carries the *glutinosa NN* genes, was crossed to Havana Seed, the necrotic-lesion reaction to the mild strain was inherited exactly as was the necrotic reaction to the severe strain, and exactly as was the reaction previously studied by Holmes (6). However, when such necrotic hosts as Clayton's TL106 and Daruma were crossed to Havana Seed, the necrotic reaction to the mild strain was inherited as a single, incompletely dominant character. In this respect, the inheritance of this latter reaction differs from that reported by Holmes (6), and also from that found by Valleau (15) for the dominant necrotic reaction of *N. tabacum* var. Judy's Pride to a white and an aucuba strain of the tobacco mosaic virus. However, Valleau's conclusion (15) that the necrotic reaction of Ambalema to the above two virus strains may prove to be an example of partial (incomplete) dominance is strengthened by the results of the present study.

The incomplete dominance of the necrotic local-lesion reaction of eggplant and of certain varieties of pepper to the tobacco mosaic virus, demonstrated by Holmes (3, 5), makes a similar mode of inheritance of such a reaction in certain *Nicotiana* hosts seem a likely possibility.

Holmes (6) designated the genes originally derived from *N. glutinosa*, which determine its necrotic local-lesion reaction to a distorting or severe strain of the tobacco mosaic virus, *NN;* and the corresponding recessive alleles present in non-necrotic *N. tabacum* varieties such as Burley, Samsoun, and Connecticut Broadleaf, *nn*. However, the results of the present study show that certain varieties of *N. tabacum* may react oppositely to two strains of this virus. Therefore, in order to determine the genotype of a given variety with respect to its reaction to the tobacco mosaic virus, at least two strain types, a mild and a severe, must be used. It is believed that the designation *nn* should be applied to the genes which determine the

reaction to the tobacco mosaic virus of such host plants as Havana Seed tobacco.

The gene pair which determines the necrotic local-lesion reactions of Clayton's TL106 and *N. tabacum* var. Daruma to the mild strain of the tobacco mosaic virus is designated $n^s n^s$. This gene pair very probably also determines the necrotic reaction of *N. sylvestris*, *N. tabacum* var. Connecticut Broadleaf, and a number of other varieties of tobacco to this strain of the virus. It is assumed that this gene pair also determines the systemic-mottling reaction of these hosts to the severe strain of the virus. The superscript s in the above designation represents the typical reactions of *N. sylvestris* to the two strains of the tobacco mosaic virus. The genes $n^s n^s$ are incompletely dominant alleles of the previously discussed *nn* genes for the completely systemic mottling reaction. The well-known *NN* genes are believed to be the dominant alleles of both the $n^s n^s$ and the *nn* genes. It is proposed that these three gene pairs are members of a series of multiple alleles in the following order, *NN*, $n^s n^s$, *nn*. For a plant to be considered as definitely carrying the $n^s n^s$ genes, its reaction to the mild strain must be inherited as an incompletely dominant character in crosses with hosts bearing the *nn* genes.

The results of the cross between Holmes Samsoun and Daruma, on which the conclusion that the *NN* factors are the dominant alleles of the $n^s n^s$ factor is based, may be considered by some as aberrant in any of several respects. First, the F_1 showed three classes of reactions when inoculated with the tobacco mosaic virus. These results have already been discussed. Secondly, with the exception of one F_3 family, definite 3:1 ratios of reaction classes were obtained in the F_2 and F_3 generations. According to Gerstel (2), an unassociated pair of *glutinosa* and *tabacum* chromosomes occurs in hybrids between Holmes Samsoun and *N. tabacum* varieties. Gametic elimination follows, and normal ratios are distorted. Thus roughly a 1:1 rather than a 3:1 ratio would be expected in the F_2 of this cross. Why such results were not obtained in the present investigation is not known. Perhaps in this particular cross, the *glutinosa* and *tabacum* pair of chromosomes usually pair and only occasionally fail to associate. This could also account for the irregular segregation of F_3 family 9 (Table 5). This interpretation has been adopted by the author as applying to this case.

It is impossible to state definitely whether or not the genes designated $n^s n^s$ also determine the necrotic reaction of *N. longiflora*, which is similar to that of *N. sylvestris*. This is because the reaction of *N. longiflora* was completely dominant over the reaction of *N. alata*, the only systemic-mottling parent with which it was crossed in this investigation.

According to Valleau (16), the N′ factors in *N. tabacum* are probably allelomorphs of the *N* factors in *N. glutinosa* carried on homologous chromosomes. Relationships between the $n^s n^s$ genes and the N′N′ genes have not been investigated. However, limited trials with a mild strain of the yellow tobacco mosaic virus (aucuba mosaic), made in the course of the present

investigation, indicate that, in the case of some varieties of tobacco, these gene symbols may be synonymous. No definite information is at hand regarding this possibility, and further work should be done in this connection. The complete dominance of the N′N′ factors as opposed to the incomplete dominance of the $n^s n^s$ factors should aid in their separation.

Nicotiana tabacum is fundamentally tetraploid. Consequently it is possible that a second pair of factors, in addition to one of the group now recognized, also influences the reaction of *N. tabacum* to the virus. In this case, the unexpressed functioning of these factors may be explained by the assumption that they are always in the dominant condition.

The results of previous workers studying other characters support the conclusions of the present investigator as to the inheritance of the necrotic reaction of *N. longiflora*. Holmes (3) obtained similar results from a cross between necrotic and non-necrotic varieties of *N. sanderae* (*N. alata*), and from one between *N. langsdorffii* (necrotic) and *N. sanderae* (non-necrotic) in reaction to a distorting, or severe strain of the virus. *N. langsdorffii* and *N. alata* belong to the same taxonomic section as does *N. longiflora*. Kostoff (10) states that the characters of the taxonomic group to which *N. longiflora* and *N. alata* belong are inherited chiefly monogenetically.

The possibility that the necrotic local-lesion reaction to the mild strain will be incorporated into non-necrotic varieties of tobacco for purposes of resistance to the tobacco mosaic virus is remote. The resistance afforded by the *NN* factors from *N. glutinosa* is far superior for this purpose because it provides resistance to the severe, as well as to the mild, strain of the virus. The chief significance of this investigation to the breeder for disease resistance lies in its emphasis on the precaution that in breeding for resistance to a virus, more than one strain of the virus should be included in routine tests.

Finally, an intriguing problem lies in the unraveling of the three types of reactions to the two virus strains exhibited by the species *N. alata*.

SUMMARY

The necrotic local-lesion reaction of *Nicotiana sylvestris*, Clayton's TL106, *N. tabacum* var. Daruma, and several other varieties of *N. tabacum* to the mild, or attenuated, green mosaic strain of the tobacco mosaic virus was incompletely dominant in the F_1 over the systemic-mottling reaction of *N. tabacum* varieties such as Havana Seed.

A single pair of incompletely dominant factors governed the necrotic reactions of TL106 and Daruma to the mild strain, as shown by 1:2:1 ratios of the three classes of reactions observed in the F_2 and F_3 generations of their crosses. That this gene pair also determines the reaction of these, and certain similarly reacting hosts, to other strains of the tobacco mosaic virus such as the mild-yellow (aucuba) mosaic strain is indicated.

In a cross with *N. alata*, the necrotic local-lesion reaction of *N. longiflora*

to the mild strain, similar to that of *N. sylvestris*, was controlled by a single pair of dominant factors.

The results of a cross involving both the $n^s n^s$ genes of Daruma and the *NN* genes of Holmes Samsoun have indicated that the latter are the dominant alleles of the former. Therefore, it is proposed that a series of multiple alleles, composed at least of *NN*, $n^s n^s$, and *nn*, exists.

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. CLAYTON, E. E. A wildfire resistant tobacco. Jour. Hered. **38**: 35–40. 1947.
2. GERSTEL, D. U. Inheritance in Nicotiana tabacum. XVII. Cytogenetical analysis of glutinosa-type resistance to mosaic disease. Genetics **28**: 533–536. 1943.
3. HOLMES, F. O. Inheritance of ability to localize tobacco-mosaic virus. Phytopath. **24**: 984–1002. 1934.
4. ———. Interspecific transfer of a gene governing type of response to tobacco-mosaic infection. Phytopath. **26**: 1007–1014. 1936.
5. ———. Inheritance of resistance to tobacco-mosaic disease in the pepper. Phytopath. **27**: 637–642. 1937.
6. ———. Inheritance of resistance to tobacco-mosaic disease in tobacco. Phytopath. **28**: 553–561. 1938.
7. ———. A comparison of the experimental host ranges of tobacco-etch and tobacco mosaic viruses. Phytopath. **36**: 643–659. 1946.
8. JOHNSON, J. A tobacco hybrid useful for virus studies. Amer. Jour. Bot. **23**: 40–46. 1936.
9. ———. Virus attenuation and the separation of strains by specific hosts. Phytopath. **37**: 822–837. 1947.
10. KOSTOFF, D. Cytogenetics of the genus Nicotiana. 1071 pp. State's Printing House, Sofia. 1941–1943. (In Russian and English.)
11. ———, and R. GEORGIEVA. II. Inheritance of necrotic reaction and plant breeding value of the form Nicotiana tabacum var. virii. *In* Resistance to tobacco mosaic virus. pp. 13–34, 46–54. Sofia. 1944. (In Russian and English.)
12. KUNKEL, L. O. Local lesions in aucuba mosaic of tomato. (Abstr.) Phytopath. **22**: 16. 1932.
13. ———. Studies on acquired immunity with tobacco and aucuba mosaics. Phytopath. **24**: 437–466. 1934.
14. SINNOTT, E. W., and L. C. DUNN. Principles of Genetics. 3d ed. 408 pp. New York and London. 1939.
15. VALLEAU, W. D. Localization and resistance to tobacco mosaic, in Nicotiana. Kentucky Agr. Exp. Sta. Res. Bul. 360: 202–230. 1935.
16. ———. The relative positions of the N and N′ factors on Nicotiana tabacum chromosomes. (Abstr.) Phytopath. **33**: 14. 1943.
17. ———, and E. M. JOHNSON. An outbreak of plantago virus in burley tobacco. Phytopath. **33**: 210–219. 1943.

EFFECT OF SEED TREATMENT ON FORAGE LEGUMES IN WET AND DRY SOIL

J. W. GERDEMANN

(Accepted for publication February 12, 1951)

The benefits obtained from chemical seed treatment of many field crops are well known, and an increasing interest is being shown in the use of chemical seed treatment on forage legumes. In greenhouse tests conducted in untreated field soil, Chilton and Garber (2) obtained highly significant increases in stands with certain fungicides and certain species of forage legumes. Other legumes did not respond to treatment and in some cases injury resulted. In a field test Allison and Torrie (1) obtained no significant increases in stands of six forage legumes treated with several seed protectants. Highly significant increases in stands of some species were obtained when treated seeds were planted in compost infested with various damping-off organisms. Germination of two species was retarded by Spergon. In greenhouse tests Cormack (3) obtained increased stands of alfalfa when the seed was treated with several fungicides. In field tests the effect of seed treatment varied, increasing the emergence in some samples but not in others. In greenhouse tests conducted in autoclaved soil, Vlitos and Preston (5) obtained highly significant increases in emergence of treated seed of a number of field legumes. In field tests only yellow hop clover showed a significant increase in emergence of treated seed. In field tests at a number of locations Kreitlow, Garber, and Robinson (4) found the response of alfalfa and red clover to seed treatment varied from decreases to small but significant increases in stand.

Seed of forage legumes is planted at a relatively shallow soil depth where rapid fluctuations in moisture may occur; if no rain falls for a few days following planting, the soil may be too dry for germination. This study compares the effect of chemical seed treatment on three forage legumes, at two moisture levels in greenhouse and field, at Urbana, Illinois, during 1949–1950.

MATERIALS AND METHODS

Red clover (*Trifolium pratense*), alfalfa (*Medicago sativa*), and white-flowered biennial sweet clover (*Melilotus alba*) were selected from the forage legumes. The fungicides tested were Arasan (50 per cent tetramethyl thiuramdisulfide), Ceresan M (7.7 per cent ethyl mercury p-toluene sulfonanilide), and Phygon XL-DDT (50 per cent 2,3 dichloro-1,4 naphthoquinone and 3 per cent dichloro-diphenyl-trichloroethane). Ten-gram samples of seed were treated with 0.25, 0.5, and 1 per cent dosage rates by weight of seeds. Five drops of a 5 per cent solution of Methocel sticker (Dow methycellulose) were added to the treated seeds to prevent loss of the fungicide dust in handling. A preliminary test with red clover showed no significant difference in emergence between untreated seed coated with

Methocel sticker and untreated seed without Methocel sticker. Following treatment the seed was stored from 12 to 15 days in paper envelopes.

The experimental design used in greenhouse experiments was a split plot with five replications. Blocks were divided into two main plots which were maintained at different soil moisture levels. The various seed treatments were assigned at random to the subplots. Each subplot consisted of two 19-in. rows 1½ in. apart. Seed was planted from ¼ to ½ in. deep, 50 seeds per row. In the dry soil the average moisture content ranged between 15 and 20 per cent; that of the wet soil was maintained between 30 and 40 per cent. Since water was applied by overhead sprinkling, the surface soil varied between greater extremes than the average. Moisture determinations were made before and after watering, from samples of soil taken from unplanted flats which received the same treatment as the main plots.

An untreated, dark brown silt loam soil was used in all greenhouse experiments. Temperatures, not controlled, varied from 55° F. to as high as 85° F. for brief periods on sunny days. Stand counts were taken when germination appeared to be complete, 16 to 23 days following planting. Since seed treatment has been reported to have no effect on post-emergence damping-off (1), a few plants that were so killed were included in the totals.

The experimental design used in field tests was a randomized block replicated 10 times. Each plot consisted of 100 seeds planted in an 8-ft. row. Rows were spaced 4 in. apart. The seed was planted 24 days following treatment, and stand counts were taken 4 weeks after planting. No post-emergence damping-off was observed.

EXPERIMENTAL RESULTS

Results of two red clover experiments in the greenhouse are summarized in table 1. No chemical treatment significantly increased the emergence of seedlings of Midland or common red clover in either wet or dry soil. In both experiments the F value for the interaction between soil moisture and seed treatment exceeded the 5 per cent level of significance, indicating a differential reaction of the various chemical treatments to soil moisture. One per cent Phygon appeared to harden the seed coats under both moisture conditions, and caused marked stunting of the plants. Arasan caused no significant increase in emergence in wet soil; in dry soil, dosages of 0.25 and 0.5 per cent appeared to cause a slight reduction in emergence. In dry soil with 1 per cent Ceresan M there was swelling of hypocotyls and roots failed to form. On common red clover, 0.25 and 0.5 per cent Ceresan M significantly reduced emergence in dry soil.

Table 1 summarizes also the results of two alfalfa experiments. With the exception of 1 per cent Ceresan M, which caused some damage to seedlings, none of the treatments had any significant effect on emergence of alfalfa. The F values for interaction between seed treatment and soil moisture were not significant in either experiment.

In tests on two sweet clover varieties (Table 1), the total emergence of common white sweet clover in wet and dry soil was significantly increased by

TABLE 1.-- Mean number of plants emerged[a] after seed treatment, in two varieties of red clover, two varieties of alfalfa, and two varieties of sweet clover planted in wet and dry soil in the greenhouse, Urbana, Illinois

Seed treatment	Dosage (per cent)	Wet soil	Dry soil	Average	Wet soil	Dry soil	Average
		Midland red clover			Common red clover		
Check	.....	62.8	62.8	62.8	76.8	72.6	74.7
Arasan	0.25	64.4	56.0*	60.2	77.8	65.8*	71.8
	0.50	62.2	55.8*	59.0	79.8	68.0	73.9
	1.00	66.6	59.4	63.0	79.6	73.4	76.5
Ceresan M	0.25	63.4	62.2	62.8	74.2	64.2*	69.2
	0.50	61.8	63.6	62.7	76.8	66.4*	71.6
	1.00	67.2	54.8*[b]	61.0	81.2	69.0[b]	75.1
Phygon	0.25	57.8	58.0	57.9	74.4	73.4	73.9
	0.50	68.8	61.0	64.9	75.6	73.8	74.7
	1.00	56.0*[b]	59.2[b]	57.6	75.8[b]	74.8[b]	75.3
LSD	0.05	6.8	6.8	.....	6.0	6.0	.....
	0.01	....	....	.....	....	....	.....
F. for treatment moisture interaction		2.38[c]			2.46		
		Ranger alfalfa			Kansas common alfalfa		
Check	.....	81.2	83.6	82.4	78.8	77.8	78.3
Arasan	0.25	82.0	85.0	83.5	78.6	81.8	80.2
	0.50	81.4	85.6	83.5	79.6	78.8	79.2
	1.00	82.4	85.2	83.8	81.4	82.0	81.7
Ceresan M	0.25	83.4	83.6	83.5	79.0	76.6	77.8
	0.50	82.2	82.6	82.4	80.8	78.6	79.7
	1.00	82.6	78.2[b]	80.4	77.2[b]	72.0[b]	74.6
Phygon	0.25	82.2	84.6	83.4	78.6	79.6	79.1
	0.50	84.0	86.2	85.1	80.2	77.8	79.0
	1.00	80.6	83.8	82.2	78.4	78.6	78.5
LSD	0.05	....	....	....	....	....	....
	0.01	....	....	....	....	....	....
F. for treatment moisture interaction		1.05[c]			0.69		
		Common white sweet clover			Evergreen sweet clover		
Check	.....	29.6	29.0	29.3	78.6	69.0	73.8
Arasan	0.25	35.0	33.4	34.2*	80.8	71.2	76.0
	0.50	32.6	34.8*	33.7*	76.2	73.8	75.0
	1.00	37.4**	32.2	34.8**	79.0	72.4	75.7
Ceresan M	0.25	34.6	33.2	33.9*	82.2	65.6	73.9
	0.50	34.8	37.2**	36.0**	76.8	63.0[b]	69.9
	1.00	29.0	27.2[b]	28.1	79.4	54.2[b]**	66.8
Phygon	0.25	30.0	31.4[b]	30.7	79.6	68.6[b]	74.1
	0.50	34.2	31.8[b]	33.0	73.2	60.8[b]*	67.0
	1.00	33.0[b]	25.6[b]	29.3	73.4	51.2[b]**	62.3
LSD	0.05	5.8	5.8	4.1	7.4	7.4	....
	0.01	7.8	7.8	5.5	9.8	9.8	....
F. for treatment moisture interaction		1.12[c]			3.46		

[a] Since the number of seeds per subplot was 100, the mean equals percentage emergence.

[b] Seedlings stunted or injured.

[c] F. value required for significance at 0.05 = 2.01; at 0.01 = 2.67.

*Difference from check is significant at the 0.05 level.

**Difference from check is significant at the 0.01 level.

all rates of Arasan and by 0.25 and 0.5 per cent Ceresan M. One per cent Ceresan M and all rates of Phygon caused subnormal growth of seedlings in dry soil, but did not significantly reduce the emergence. The F value for interaction between seed treatment and soil moisture did not show significance in this experiment. However, the calculations were based on the number of plants emerged and did not take into account the fact that some treatments caused injury to seedlings in dry soil without reducing the emergence. The variety Evergreen did not respond to Arasan treatment; in dry soil 0.5 and 1 per cent Ceresan M and all rates of treatment with Phygon either injured the seedlings or reduced the emergence, or both. The F value for interaction between seed treatment and soil moisture in this experiment exceeded the 1 per cent level of significance.

Results of field experiments on red clover, alfalfa, and sweet clover are in table 2. Because of a late spring, these tests were planted later than the

TABLE 2.-- Mean number of plants emerged after seed treatment, in the field stand of three forage legumes on the Agronomy south farm, Urbana, Illinois

Seed treatment	Dosage (per cent)	Common red clover	Kansas common alfalfa	Common white sweet clover	Willamette sweet clover
Check	.....	24.7	50.2	9.7	16.8
Arasan	0.25	23.1	44.0	6.5	11.4**
	0.50	19.8	51.7	7.5	11.6**
	1.00	16.7**	56.1	6.3	12.1**
Ceresan M ..	0.25	23.6	49.8	....	.....
	0.50	23.8	42.8	....	.....
Phygon	0.25	22.3	45.1	....	.....
	0.50	19.1*	47.1	....	.....
	1.00	15.2**	41.0*	....	.....
LSD	0.05	5.2	7.5	....	2.6
	0.01	6.9	9.9	....	3.5

*Difference from check is significant at 0.05 level.
**Difference from check is significant at 0.01 level.

usual planting time for spring-seeded legumes. One per cent Ceresan M was excluded from the field tests and only Arasan was tested on sweet clover. The seed in these experiments was planted May 11 in moist soil. No rain fell for 10 days following planting, but during the next 15 days rainfall was 1.5 inches.[1] The average daily temperature of the first inch of soil ranged from 55° F. on the date of planting to 69° F. on the date the final counts were made. The average soil temperature for the entire period was 62.4° F.

None of the treatments caused a significant increase in emergence over the check and several caused significant reductions. The emergence of red clover was significantly reduced by 1 per cent Arasan, and 0.5 per cent and 1 per cent Phygon. One per cent Phygon caused a significant reduction in emergence of alfalfa. Emergence of all sweet clover was low. Emergence

[1] Weather records taken by the Illinois Water Survey at the Agronomy south farm.

of Willamette sweet clover was significantly reduced by all rates of Arasan treatment. Emergence of common sweet clover treated with Arasan was lower than the check, but not significantly so.

DISCUSSION AND SUMMARY

The effect of three chemical seed treatments on red clover, alfalfa, and sweet clover was studied in wet and dry soil. Red clover seed treated with three different rates of Arasan, Ceresan M, and Phygon, and planted in wet and dry soil showed no significant increase in emergence over untreated seed. Some treatments which appeared harmless in wet soil reduced the emergence or injured the seedlings when the seed was planted in dry soil.

Alfalfa was neither benefited nor injured by most treatments. The reaction to treatment was nearly the same in wet and dry soil. One per cent Ceresan M caused injury to seedlings in wet and dry soil in greenhouse tests, and 1 per cent Phygon reduced the emergence in the field under drouth conditions.

Sweet clover emergence was increased by treatment with Arasan and some of the lower rates of Ceresan M used in greenhouse tests. One seed lot was not benefited by any seed treatment. A number of treatments which were harmless in wet soil reduced the emergence or injured the seedlings in dry soil. Arasan, which appeared beneficial or harmless in dry soil in greenhouse tests, significantly reduced the emergence of sweet clover in the field under drouth conditions.

Final recommendations for seed treatment of forage legumes should be based on experiments conducted in many soil types and under varied environmental conditions. Beneficial results have been obtained from seed treatment of some forage crops when the seed was planted in compost or soil infested with damping-off organisms (1, 4) or when the seed was infected with a pathogenic organism (3). The value of seed treatment may depend on how frequently similar conditions occur in the field. If, however, a fungicide becomes injurious under some environmental condition, the frequency of occurrence of this condition also becomes a factor in determining whether, over a period of years, the net result will be benefit or injury.

DEPARTMENT OF AGRONOMY, UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

LITERATURE CITED

1. ALLISON, J. LEWIS, and J. H. TORRIE. Effect of several seed protectants on germination and stands of various forage legumes. Phytopath. **34**: 799–804. 1944.
2. CHILTON, S. J. P., and R. J. GARBER. Effect of seed treatment on stands of some forage legumes. Jour. Amer. Soc. Agron. **33**: 75–83. 1941.
3. CORMACK, M. W. Studies on Ascochyta imperfecta, a seed- and soil-borne parasite of alfalfa. Phytopath. **35**: 838–855. 1945.
4. KREITLOW, K. W., R. J. GARBER, and R. R. ROBINSON. Investigations on seed treatment of alfalfa, red clover, and Sudan grass for control of damping-off. Phytopath. **40**: 883–898. 1950.
5. VLITOS, A. J., and D. A. PRESTON. Seed treatment of field legumes. Phytopath. **39**: 706–714. 1949.

SILKY THREADBLIGHT OF ELAEAGNUS PUNGENS CAUSED BY RHIZOCTONIA RAMICOLA N. SP.[1]

GEORGE F. WEBER[2] AND DANIEL A. ROBERTS

(Accepted for publication February 20, 1951)

Observational and experimental data are herewith presented concerning silky threadblight, a new disease of the perennial ornamental, *Elaeagnus pungens*.

THE DISEASE

Host range. Silky threadblight of *Elaeagnus pungens* was first observed in isolated areas of a shrub border-planting during the summer of 1945 in Gainesville, Florida. Natural infections occur on *E. pungens, Ilex crenata, Erythrina herbacea, Pittosporum tobira, Lagerstroemia indica,* and *Feijoa sellowiana.* Inoculation experiments indicate that *Gelsemium sempervirens, Rhododendron indicum,* and *Pyracantha formosana* are susceptible, whereas *Nerium oleander* and *Ligustrum lucidum* did not become diseased. An extensive survey for the disease has not yet been made, and the current list of susceptible plants is not long.

Distribution and importance. The disease has been found only in Gainesville, Florida. All reported hosts are grown extensively in the southeastern part of the United States from Virginia southward, where development of the disease would be favored by prolonged rainy periods and continuous high humidity and high temperature. The disease occurs annually, is of minor economic importance, and does not spread rapidly.

Symptomatology. The disease, unassociated with the soil as to origin, has been observed affecting isolated areas in shrub plantings, involving tender twigs, petioles, and leaf blades. During warm and humid weather, short, delicate, gray to olivaceous hyphal strands develop and extend across leaf axils, and grow along the surfaces of twigs as far as 20 mm. above and below the bases of infected petioles. Dead leaves may be matted together or held dangling from the twigs by these fungus threads. Denser mats of hyphae usually appear at the point of contact of diseased and healthy leaf blades, binding them together (Fig. 1 and 2).

Lesions on leaf blades may occur at any point which is in contact with external mycelium, and appear at first as small, necrotic spots, enlarging slowly, occasionally involving the entire blade. Ordinarily, lesions reach a maximum diameter of about 25 mm., and are characterized by tan necrotic centers, purplish-brown margins, and brittle texture. (Fig. 1.) Under slight mechanical pressure, necrotic areas crack and fall away. When only

[1] From a thesis presented by the junior author to the Graduate School of the University of Florida, in partial fulfillment of the requirements for the Master of Science degree.

[2] Professor of Plant Pathology, College of Agriculture, University of Florida, Gainesville, Florida.

a portion of the blade is infected, defoliation does not occur, and diseased leaves persist throughout the autumn and winter seasons. During these months, the tan lesions are conspicuous symptoms of the disease. Infection of petioles results in discoloration, followed by cortical necrosis and sloughing away of the diseased tissues.

No sclerotia have been found.

FIG. 1. Silky threadblight of *Elaeagnus pungens*. Left, lesions on green leaves and brown, dead leaves held in place by surface hyphae. Right, lower sides of leaves covered with hyphae; leaves at top are healthy.

THE FUNGUS

Taxonomy. According to Duggar (2), many *Corticium* spp. and *Rhizoctonia* spp. are soil-inhabiting organisms capable of living from season to season in the form of mycelia or sclerotia in diseased tissue, decaying plant debris, or in the soil. The parasitic vegetative stages of these fungi are closely associated with the soil, and the symptoms of the diseases caused by them occur on plant parts located near the soil line. Only the saprophytic sexual stages occur aerially, usually not more than 10 to 25 cm. above the surface of the soil. Although an extensive host range for *R. solani* has been demonstrated, no report of a disease of *Elaeagnus pungens* caused by this fungus has been discovered in literature. The *Rhizoctonia* sp. from *Elaeagnus* occurs only on aerial plant parts and is in no way associated with the soil. Sclerotia have not been observed in nature or in culture. Consequently, the fungus is considered separate and distinct from the several soil-inhabiting *Rhizoctonia* spp. and *Corticium* spp.

A second group of parasitic *Rhizoctonia* spp. and *Corticium* spp., described by Burt (1), Matz (4), Voorhees (5), and Weber (6), includes the aerial forms *C. stevensii* Burt, *C. koleroga* (Cooke), v. Höhn., *C. microsclerotia* (Matz) Web., and *Rhizoctonia zeae* Voorh. Burt (1) characterizes

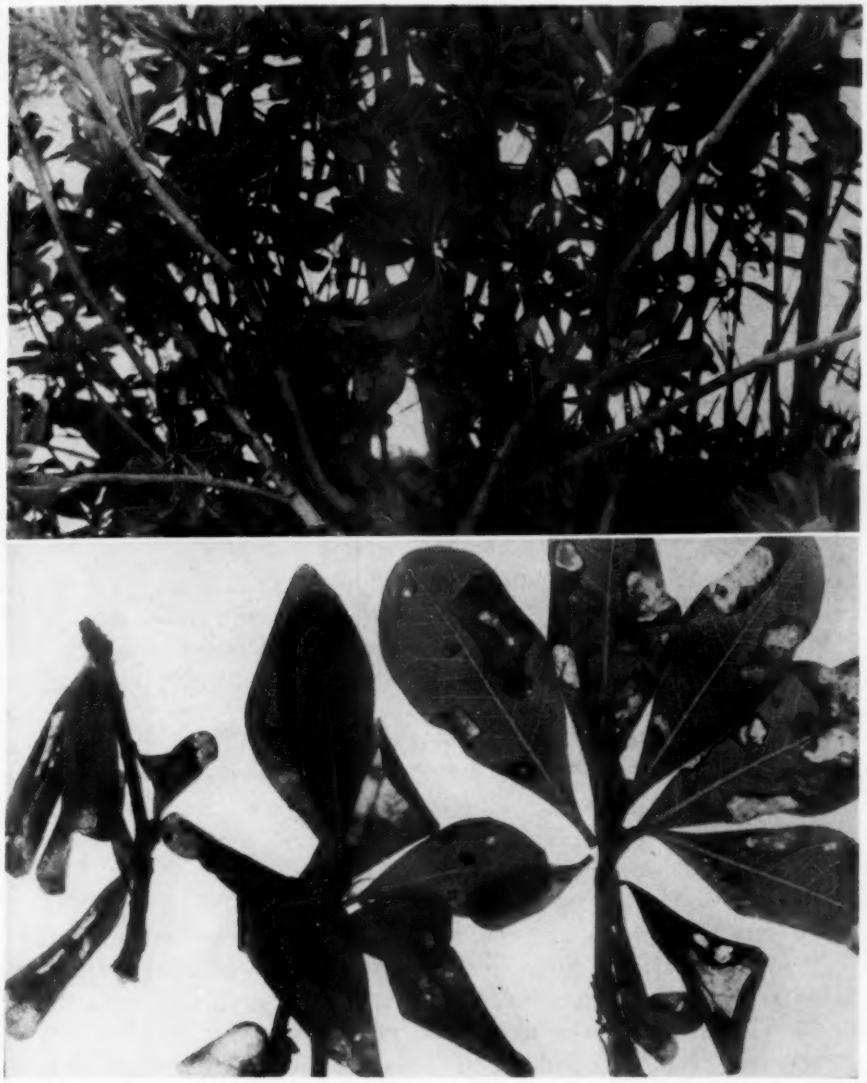

FIG. 2. Silky threadblight of *Pittosporum tobira*. Above, severe infection and defoliation. Below, right to left, progressive stages in the development of the disease.

another aerial form, *C. salmonicolor* Berk. & Broome, by the incrustation of a pink hymenium occurring on diseased limbs. The absence of sclerotia or a pink hymenium distinguishes the *Rhizoctonia* sp. attacking *Elaeagnus pungens* from all of these aerial fungi except *Corticium koleroga*. The short, fine, filamentous, olivaceous-brown mycelial threads distinguish it

from *C. koleroga*, which forms long, more thickened, fuscous threads. Because of these distinguishing characteristics, this fungus will be described as a new species. The following binomial is proposed:

Rhizoctonia ramicola n. sp.

Sclerotiis absentibus; mycelio aerio, primum hyalino, granuloso dein casso, in culturis primum olivaceo-cineraceo dein purpuraceo-brunneo; hyphis septatis, 3.5–7.7 × 22.0–155.0 μ, ad septa constrictis, angulis ramorum primum acutis dein rectis; septis nodosis absentibus.

Hab. in ramis et foliis vivis *Elaeagni pungentis* Thun., Gainesville, Florida, Am. Bor.

Sclerotia absent; vegetative mycelium aerial, hyaline and granular at first, empty at maturity, forming delicate threads in leaf axils, in culture olivaceous-gray at first, becoming purplish-brown with age; hyphae septate, 3.5–7.7 μ wide, 22.0–155.0 μ between septa, constricted at septa, branching at acute angles when young, at right angles at maturity, the first septa of lateral hyphae formed 5.0–5.8 μ from main hyphae; clamp connections lacking.

On living twigs, petioles, and leaf blades of *Elaeagnus pungens*, Thun., Gainesville, Florida, U. S. A.

Dried specimen deposited in the University of Florida Agricultural Experiment Station Herbarium, number F-41694.

Morphology. On 2 per cent potato-dextrose agar, the young hyphae branch at acute angles, while mature hyphae branch at right angles. Branches are constricted at the bases. Mycelial growth forms a dense mat in culture, and individual hyphae penetrate the substrate slightly. No sclerotia are formed. A lavender tint in mature cultures is a diagnostic characteristic of the species.

Physiology. The effect of temperature upon the rate of growth in culture of mycelium transfers of *Rhizoctonia ramicola* and *R. solani* was studied by measuring the diameters of colonies on three plates of 2 per cent potato-dextrose agar infested with each isolate and incubated for 72 hr. at temperatures ranging from 10° to 40° C.

Although the maximum growth of each isolate occurred near 29° C., the rate of growth of the potato isolate was distinctly slower, and its growth occurred over a greater range in temperature. *R. ramicola* grew less rapidly than *R. solani* from potato at 24° C. and below, but more rapidly above 24° C. The conditions in nature observed to be most favorable for the development of *R. ramicola* are temperatures of 25°–30° C., and relative humidities of 75 per cent and above. The absence of either or both of these conditions results in retarded growth and reduced severity of the disease. Incubation at 37° C. for 72 hr. killed both organisms tested.

Plates were poured from tubes of pH-adjusted agar, and when this hardened, were infested with mycelium transfers of *R. ramicola* and incubated for 72 hr. at 28°–30° C.; diameters of mycelial growths were then measured.

The optimum pH for growth on 2 per cent potato-dextrose agar is near 6.3, although the fungus tolerates ranges of acidity and alkalinity from between pH 3.4 and 4.1 to a point between pH 9.3 and 10.0. At the conclusion of the experiment, tests showed that the pH of all cultures had adjusted toward neutrality, with the exception of the one at pH 7.1, which remained unchanged. The organism developed equally well in total darkness and in intermittent, subdued, and continuous bright light.

Pathogenicity. The causal organism was present wherever the disease was observed, and the fungus was isolated in pure culture from twigs, petioles, and leaf blades of infected host plants. In order to determine some factors governing the pathogenicity of *R. ramicola* upon *Elaeagnus pungens*, inoculation experiments were conducted under temperatures varying from 23° to 34° C., and on plants out of doors, in the greenhouse, and in moist chambers. All inoculated plants became infected when tempera-

TABLE 1. -- Percentage emergence and percentage damping-off of seedlings grown in sterilized soil infested with Rhizoctonia ramicola and R. solani

Fungus	Emergence			Damping-off[a]		
	Beans	Cabbage	Lettuce	Beans	Cabbage	Lettuce
None	95	67	83	0	1.5	2.4
R. ramicola	90	63	84	0	0	1.2
R. solani from						
beans	62	27	15	19	52	39
lupine	75	37	34	10	42	29
trifoliata	66	15	20	15	44	50
oat	78	34	30	18	52	45
pine	85	64	83	0	1.6	2.4

[a]Based upon emerged seedlings.

tures about 30° C. were accompanied by high relative humidity and an abundance of inoculum. *R. ramicola* was reisolated from petioles, twigs, and leaf blades of inoculated plants.

The pathogenicity of *R. ramicola* and several isolates of *R. solani* to bean, cabbage, and lettuce seedlings was tested. Sterilized soil was placed in flats in the greenhouse and infested with finely chopped mycelia which had been grown on 2 per cent potato-dextrose agar. In each flat were planted 25 bean, 100 cabbage, and 100 lettuce seeds. Each treatment was duplicated, and flats of noninoculated soil were seeded to provide controls. Seedlings were counted and observed for 10 days following emergence (Table 1).

Under the conditions of this experiment, isolates of *Rhizoctonia solani* from bean, lupine, oat, and trifoliata (*Poncirus trifoliata*) caused seed decay and damping-off of seedlings of bean, cabbage, and lettuce. *R. ramicola* and the *R. solani* isolate from pine failed to retard or prevent germination of seeds, and caused no significant damping-off of seedlings.

HOST AND FUNGUS RELATIONS

Seasonal development of the disease. Most active growth of the fungus and most rapid development of the disease occur when warm, humid conditions prevail. In the Gainesville, Florida, area weather conditions optimum for the development of the disease have been observed during July, August, and September, although infection may occur at any time between May and October. During the other months of the year, the fungus remains more or less dormant, reviving upon the return of favorable temperature and moisture conditions as indicated by the annual recurrence of the disease in the same locations.

Observations of the disease in nature and results of inoculation experiments have shown that infection may occur whenever daytime temperatures between 24° and 32° C. and mean relative humidity near 75 per cent have persisted for 5 or 6 days. Under optimum conditions, symptoms of the disease appeared within 4 to 9 days following inoculation, 80 per cent of the infection appearing within 7 days. Leaf lesions generally became apparent after 4 days, and complete destruction of petiole cortical parenchyma occurred 7 to 9 days after inoculation.

Rhizoctonia ramicola has been observed under many field conditions, but a perfect stage has not been discovered. All attempts to produce vegetative spores or a sexual stage under controlled conditions have failed.

Silky threadblight of *Elaeagnus pungens* appears annually in previously infected plantings, indicating that the fungus overwinters in diseased tissues or as external hibernating hyphae. The fungus has been isolated from 7-month-old lesions on leaves which had remained alive throughout the winter.

Pathological anatomy. Hyphae penetrate the petioles through lenticels and the basal cells of scales. Microscopic examination of cross sections of diseased petioles revealed intercellular hyphae in the cortical parenchymatous tissues. The means by which the fungus destroys these cells have not been determined, although it appears that the process is similar to that of *R. solani* as demonstrated by Matsumoto (3). It is suggested that mechanical pressure and enzymatic activity break and dissolve the cell walls, thereby allowing the parasite to absorb the contents. The fungus does not break down the woody cells of the central cylinder, but infected cortical cells are killed and slough off easily.

Penetration through stomata gives the fungus ready access to the spongy mesophyll of leaf blades. In free-hand sections through diseased leaf blades, intercellular hyphae were observed throughout the mesophyll.

CONTROL RECOMMENDATIONS

One or more summer applications of a copper-containing fungicide made on diseased and adjacent plants is recommended for control of the disease in plantings where it is already established. Distribution of the pathogen

may be prevented through the practice of careful examination of nursery stock and the removal and destruction of infected plants or plant parts.

SUMMARY

The heretofore undescribed silky threadblight of foliage of *Elaeagnus pungens* in Florida is caused by the fungus *Rhizoctonia ramicola* n. sp. The diagnostic symptoms and signs of the disease are cortical necrosis of petioles, necrotic leaf lesions, and the attendant superficial mycelial strands. The causal organism is characterized by its aerial habitat, failure to live in the soil, absence of spores and sclerotia, and lavender color of mature mycelium on potato-dextrose agar. The fungus is pathogenic to species of *Elaeagnus, Ilex, Erythrina, Pittosporum, Lagerstroemia, Feijoa, Gelsemium, Rhododendron,* and *Pyracantha.* Optimum growth occurred in media adjusted to pH 6.3 and held at 29° C.

PLANT PATHOLOGY DEPARTMENT, UNIVERSITY OF FLORIDA
GAINESVILLE, FLORIDA

LITERATURE CITED

1. BURT, E. A. The Thelephoraceae of North America XV. Ann. Mo. Bot. Gard. **13**: 173–354. 1926.
2. DUGGAR, B. M. Rhizoctonia crocorum (Pers.) DC., and R. solani Kühn (Corticium vagum B. & C.), with notes on other species. Ann. Mo. Bot. Gard. **2**: 403–458. 1915.
3. MATSUMOTO, T. Further studies on physiology of Rhizoctonia solani Kühn. Imperial Coll. Agr. & For. Morioka Bul. **5**: 1–64. 1923.
4. MATZ, J. A Rhizoctonia of the fig. Phytopath. **7**: 110–118. 1917.
5. VOORHEES, R. K. Sclerotial rot of corn caused by Rhizoctonia zeae n. sp. Phytopath. **24**: 1290–1303. 1934.
6. WEBER, GEORGE F. Web-blight, a disease of beans caused by Corticium microsclerotia. Phytopath. **29**: 559–575. 1939.

INFLUENCE OF PREINOCULATION ENVIRONMENT ON THE INFECTION OF BARLEY AND WHEAT BY POWDERY MILDEW[1]

V. F. TAPKE[2]

(Accepted for publication February 28, 1951)

The role of environment in the epidemiology of powdery mildew (*Erysiphe graminis* DC.) of the small grains has long been a baffling one. Unlike any other plant disease, apparently, powdery mildew has been reported in epidemic proportions in seasons of wet, dry, cool, and hot weather. Surprisingly, after years of existence in the United States as a disease of relatively minor importance, powdery mildew of barley and wheat lately has become highly destructive in the Southeastern and Atlantic Coast States. Recent studies (13, 53) indicate that this outbreak is due to new physiologic races of the pathogen. A concerted move already is under way to isolate races of the parasite, and to breed wheats and barleys resistant to the different races as a means of control. Doubtless this work can be expedited if the present hazy knowledge concerning environmental conditions that promote mildew is clarified. The present investigation was undertaken to this end. Abstracts of some of the results reported herein have been published (50, 51, 52).

LITERATURE REVIEW

The literature on small-grain powdery mildew indicates that the pathogen has the capacity to persist over a wide range of climatic conditions. The conidia, largely responsible for dissemination of the fungus, germinate at atmospheric humidities from 0 to 100 per cent (6, 8, 59) and at temperatures from $-2°$ to 35° C. (8, 37). Infection of wheat by *Erysiphe graminis tritici* has been obtained at from 2° to 30° C. (37), and mildew on small-grain plants kept in a greenhouse during the summer months survived temperatures up to 43° C. so long as there were alternating cooler periods (8). Corner (10) noted that mildew thrived during a hot, dry summer in England when other parasitic fungi suffered from drought; Montemartini (33) found mildew active in winter in Italy; and both of these observations are in accord with those Cherewick (8) has made in Manitoba, Canada.

A fact of further interest is the unanimous agreement among numerous observers that mildew in the various small grains is more severe in fields, or spots in fields, where the stand is thick and the plants have developed a lush, rank, tender growth (1, 3, 4, 5, 7, 32, 35, 36, 39, 41, 42, 43, 58). There is likewise agreement that nitrogenous fertilizers, long known as effective in

[1] Cooperative investigations of the U. S. Department of Agriculture and the New York (Cornell) Agricultural Experiment Station.

[2] Plant Industry Station, Beltsville, Maryland.

promoting a tender, leafy growth, also promote susceptibility (1, 17, 19, 28, 31, 35, 43, 48, 54, 58). In contrast, wide differences of opinion prevail relative to the kind of environment that promotes mildew. For example, the following conditions have been held conducive to high infection: wet (9, 12, 26, 59); wet and cool (25, 27, 32); cool (2); cool, humid and cloudy (11); heavy dews and abundant sunshine (56); frequent rains and humid weather (41); warm and moist (7); relatively dry (8); dry (22, 24, 34, 35, 49, 58); hot and dry (10, 14); atmospheric humidity of 100 per cent (17, 29), 95 per cent (57), 75–80 per cent (15); light of moderate duration and intensity (8, 18, 20, 21, 29, 38, 40, 47, 55, 56, 57, 60); conditions upsetting the balance between uptake of water and transpiration (23); a period of flaccidity or partial wilting of the host (43, 44, 45, 46); and preinoculation conditions promoting lush, tender growth of the plants (50, 51, 52).

EXPERIMENTAL RESULTS

A striking fact brought out by the literature review above is the wealth of positive evidence from many sources that (a) mildew in the various small grains is more severe in fields or spots in fields where the plants have developed a rank, tender growth, and (b) that nitrogenous fertilizers promote susceptibility. This led the writer to suspect that the tender tissue of well-fed, rank-growing plants might predispose them to infection through supplying the fungus with abundant food easily obtained. In order to determine this, plants were grown under temperate conditions to produce tender tissue, and under cold or dry conditions to produce non-tender or "toughened" tissue. In the first experiment, seed of the susceptible Oderbrucker (C. I. 4666) and White Smyrna (C. I. 910) barleys was sown September 5, 1946, in 6-in. pots in a greenhouse at Beltsville, Maryland, maintained at 16°–21° C. (60.8°–69.8° F.). Ten days after seedling emergence, one-third of the pots of each variety were put outdoors. The remaining pots were kept in the greenhouse and treated alike except that one-half of those of each variety were watered liberally every day, the other pots receiving only an occasional light watering, just enough to keep the plants alive and growing slowly. On November 4, after approximately 6½ weeks under the different conditions, the plants reflected their environments sharply as shown in figure 1. At that time, all of the plants of both varieties, both from outdoors and indoors, were brought together on a greenhouse bench and inoculated: heavily mildewed plants were shaken over them, special care being taken to insure uniformity in the distribution of inoculum. Thereafter, all of the plants, approximately 100, were watered uniformly and liberally, kept in a greenhouse at moderate temperatures, and reinoculated each week. Striking differences in susceptibility became apparent within 2 weeks after the first inoculation, as shown in figure 2. Very little mildew developed on the short, heavily tillered plants grown outdoors. The greenhouse-grown plants lightly watered before inoculation developed a moderate amount of mildew. The tall, succulent, liberally watered green-

house plants were attacked severely. About 3 weeks after the first inoculation, the plants that had been outdoors and had grown very rapidly under the temperate greenhouse conditions gradually began to lose their resistance. By 1 month after the initial inoculation these plants had become very susceptible. The plants that had been grown in the greenhouse and lightly watered before inoculation lost the moderate degree of their initial resistance and became highly susceptible. The plants that had been grown continuously in the greenhouse and watered liberally were highly susceptible

FIG. 1. Representative plants of Oderbrucker barley 8½ weeks old in early November, after exposure for 6½ weeks to different environments: in a greenhouse at 60°–70° F. and watered liberally (A) or watered lightly (C), and outdoors under chilly autumn conditions (B).

at all times. The two varieties used in the test reacted similarly except that Oderbrucker proved somewhat more susceptible than White Smyrna.

This first experiment produced clear evidence that environmental conditions under which barley plants are grown before inoculation may materially influence their reaction to mildew immediately and for some time after inoculation.

In the autumn of 1947, the experiment was repeated with the same two varieties except that the plants were inoculated after exposure to the three

different conditions for 11 weeks instead of only 6½ weeks as in the previous year. In the autumn of 1948 the varieties Excelsior (C. I. 1248), Freja (C. I. 7130), Hannchen (C. I. 531), Trebi (C. I. 936), and five unnamed mildew-susceptible spring barleys (C. I. 7154, C. I. 7156, C. I. 7247, C. I. 7322, and C. I. 7558) were inoculated after 2 months' exposure to the three different conditions. The results in both 1947 and 1948 were positive and similar to those of 1946 in all respects.

In the autumn of 1949, the foregoing procedures used in the study of barley were applied to the spring wheats Huron (R. L. 20), Pentad (C. I. 3320), and Thatcher (C. I. 10003). After exposure to the three different

FIG. 2. Susceptibility to mildew in the top four leaves from each of three Oderbrucker barley plants inoculated in November 1946, after growth for 6½ weeks in different environments: grown in a greenhouse at 60°–70° F. and watered liberally (A) or watered lightly (B), and grown outdoors under chilly autumn conditions (C).

environmental conditions for 8 weeks, the varieties were inoculated uniformly and then kept in a greenhouse under temperate conditions favorable to infection. As with barley, the wheat plants grown in the greenhouse and watered liberally before inoculation proved the most susceptible, the greenhouse plants lightly watered before inoculation were somewhat less susceptible, and the plants grown outdoors before inoculation developed relatively little mildew. The resistant status of the outdoor plants, as with barley, was well maintained for 3 to 4 weeks, then rather rapidly gave way to susceptibility equal to that of the other plants.

Graf-Marin (16) found that leaves of mature barley plants were more resistant to mildew and had a thicker cuticle (2.5–5.0 μ) than did leaves of

young plants (0.4–1.5 μ). The mature leaves became susceptible, moreover, when the thickened cuticle was removed. Whether cuticle thickness or some other factor or factors are involved in the predisposition of barley and wheat to powdery mildew remains to be determined.

REACTION OF SEEDLINGS

Preliminary studies of seedlings have indicated that their reaction to preinoculation environment may differ from that of older plants. In one test, for example, a flat of nearly 100 Oderbrucker seedlings about ½ in. high was placed outdoors at Beltsville, Maryland, in mid-January, 1949. After exposure to the cold weather for 3 weeks the flat was brought into a greenhouse maintained at 60°–70° F. At this time the soil in the flat was frozen solid and only the tips of the seedlings protruded above the snow that covered the flat. Six hours later the seedlings were inoculated, as were Oderbrucker seedlings of similar height that had been grown continually in the greenhouse from seed sown 9 days previously. The seedlings exposed to the cold weather were as susceptible as those continually exposed to the mild greenhouse conditions. Similar results have been obtained in several later experiments. This may account for the frequent heavy attacks of powdery mildew on fall-sown barley despite cold weather. A high susceptibility in seedlings has been noted by a number of investigators. Further study of this phase of the mildew-environment relationship is needed.

COMPLEXITY OF INFECTION

In the foregoing tests, only variously preconditioned mildew-free plants were tested, and the observations were confined largely to their behavior after the first inoculation. Under field conditions mildew infection, once started, usually produces many successive crops of conidial inoculum for successive inoculation of the host plants throughout the growing season. Therefore, at least three environmental influences apparently come into play at the moment of each inoculation: (a) Influence of preinoculation environment on the plant's predisposition, (b) influence of immediate post-inoculation environment on infection, and (c) influence of post-inoculation environment in conditioning the plant to subsequent infections. It would appear that mildew infection is hardly the simple phenomenon it has long been considered to be.

VARIATIONS

In all of the foregoing experiments, plants subjected to cold weather conditions before inoculation, as noted above, were less susceptible to mildew than were liberally watered plants grown in a temperate greenhouse before inoculation. The differences, however, were not always so great as those shown in figure 2. In several tests with the extremely susceptible Little Club (C. I. 4066) wheat in the autumn of 1949, the reduction in mildew as a result of preinoculation exposure to outdoor conditions was only slight.

Variation in degree of response to outdoor conditions was observed in other varieties. Also the plants exposed to outdoor conditions before inoculation appeared to be more resistant after the relatively cold autumns of 1946 and 1948 than after the milder autumns of 1947 and 1949 at Beltsville, Maryland.

In the foregoing studies it was also observed that the incidence of mildew on greenhouse-grown plants lightly watered before inoculation varied more than that on plants in the other treatments. Also, at the time of inoculation, the color of these plants frequently was a darker, richer green than that of the liberally watered plants grown in the greenhouse. In a study of this variation, Oderbrucker barley plants were grown under the three different conditions as usual, but the plants were potted in soil of low fertility instead of the fertile soil previously used. The result was that the plants grown under chilly outdoor conditions for several months before inoculation became lightly infected as usual, but the greenhouse-grown plants that were only lightly watered before inoculation developed nearly as much mildew as did those that were liberally watered before inoculation. Evidently, during the preinoculation period, the small amount of plant food contained in the low-grade soil had been leached from the liberally watered pots and more or less conserved in the lightly watered pots to become available when the usual uniform rate of liberal watering was applied to all pots after the plants were inoculated.

These results appear to shed new light on an interesting phase of the powdery mildew-environment relationship. As noted earlier, it has been difficult to understand the numerous and world-wide reports of powdery-mildew epidemics in the small grains in seasons of widely different climatic conditions. The results above indicate that even in the presence of a conspicuous environmental factor such as drought in the foregoing test, the plants still may be highly susceptible to mildew. Evidently the critical factor in determining the degree of plant predisposition to powdery mildew is not necessarily a predominant or conspicuous type of weather that stamps the season as being hot, cool, wet, dry, etc., but rather, the condition of the plants as determined by the interaction of all factors involved in growth. In a so-called dry season, for example, the condition and mildew susceptibility of plants growing in cool, fertile soil doubtless would differ from that of plants growing in hot, impoverished soil. It would seem possible, therefore, to have epidemics of mildew in seasons of different kind, assuming that the mildew fungus has the capacity to produce infection over the wide range of climatic conditions noted in the foregoing literature review.

The confusion inherent in associating an unusually low or high incidence of mildew with a conspicuous weather condition in some season was strikingly exemplified in 1947. The spring was unusually cool and wet in many parts of the country, including Ithaca, New York, and Manhattan, Kansas. At Ithaca there was much less mildew on wheat and barley than in any of the 13 preceding years, during which the writer observed cereal diseases on the numerous plots of the Department of Plant Breeding at Cornell Univer-

sity. At Manhattan, Kansas, however, Johnston (25) reported that the long, cool, wet spring of 1947 was highly favorable to the development of *Erysiphe graminis tritici* and that never before had he seen wheat mildew so general and so severe.

APPLICATION OF THE KNOWLEDGE OF ENVIRONMENTAL PRECONDITIONING TO THE RESULTS OF RIVERA

A unique phase of the mildew-environment complex that hitherto has been difficult to understand is embodied in the reports of Rivera (43, 44, 45, 46). Wheat plants grown in a greenhouse and plants grown outdoors were placed under a bell jar "full of moist air and containing a large number of spores." The bell jar was then exposed suddenly to direct sunlight. The greenhouse-grown plants wilted and eventually succumbed to mildew. The plants that had been grown outdoors didn't wilt and didn't take mildew. Rivera therefore concluded that the "moment of least resistance" occurs during a period of flaccidity or partial wilting of the host tissues, and that "green organs at the height of their maximum turgidity prove themselves to be relatively or absolutely resistant." Cherewick (8), however, found that mildew developed equally well on turgid and wilted plants. Mackie (30) reported heavy infections on turgid plants, and the writer repeatedly has obtained very severe mildew on plants kept continually in the turgid state in moist chambers.

In a study of wilting as a factor influencing susceptibility, the writer conducted five tests with the highly susceptible Oderbrucker, Odessa, and Trebi barleys. All plants used were grown in pots. Prior to inoculation, tender greenhouse plants were put into two groups. One continued to receive the usual daily watering while the other was left without water for 3 to 5 days or until the leaves became thoroughly limp and droopy. Also toughened plants of the same age as the above but grown under the outdoor conditions of November and December 1949 at Beltsville, Maryland, were brought into the greenhouse and likewise watered or subjected to drought until wilted. In two of the experiments the outdoor plants receiving the dry treatment also were subjected to warm air from an electric heater for 1 hr. before inoculation to increase the wilting. Wilted and nonwilted plants then were inoculated simultaneously, and kept in a temperate greenhouse thereafter. Upon inoculation the wilted plants were watered immediately or 8, 16, or 24 hr. thereafter. The nonwilted plants were watered daily and the plants remained fully turgid throughout the tests. It was found that the tender, greenhouse-grown plants became heavily mildewed and the toughened plants grown outdoors before inoculation developed only a light infection, regardless of whether or not the plants were wilted at the time of inoculation. The results were definite, therefore, in showing that wilting had no apparent effect on susceptibility, and that the reactions of the plants merely reflected the environmental conditions to which they had been exposed before inoculation. In view of these results it would seem

that: 1) The greenhouse-grown plants in Rivera's studies succumbed to mildew because they were tender and succulent; 2) their initial flaccidity was an incidental phenomenon due to sudden exposure of tender tissue to heat that penetrated the bell jar when the latter was suddenly exposed to the sun; and 3) the outdoor plants in Rivera's tests did not wilt or succumb to mildew because they had been preconditioned by the outdoor environment.

SUMMARY

The present research has brought to light a factor, hitherto unsuspected apparently, that plays a major role in mildew epidemiology: the environmental conditions to which plants are exposed before inoculation may greatly influence their reaction to mildew. Susceptibility in susceptible varieties is enhanced by preinoculation conditions that promote a lush, tender growth, such as moderately cool, moist air, ample watering, moderate intensity of light, and liberal addition of nitrogenous fertilizer to the soil. Resistance is promoted by preinoculation conditions that "toughen" the plants, such as exposure to cold, heat, drought, and long intense light.

After exposure to chilly autumn outdoor conditions at Beltsville, Maryland, for 6½ to 11 weeks, susceptible barleys and wheats growing in pots were brought into the greenhouse, inoculated, and kept thereafter under temperate conditions. These plants usually became only lightly infected, whereas plants of the same age but grown continually in the greenhouse usually became very heavily mildewed. The toughening or other conditioning effects of the rugged, outdoor preinoculation period were retained for 3 or 4 weeks, after which the plants became as susceptible as those continually grown under temperate conditions. Varieties differed in degree of reaction to environment.

The reaction of young seedlings to preinoculation environment may differ from that of older plants. In several tests seedlings exposed to cold weather for a month, then inoculated and kept under temperate conditions, were as highly susceptible as were seedlings of similar size from a later seeding and not exposed to cold.

The present results indicate that the degree of plant predisposition to powdery mildew is determined not necessarily by a predominant or conspicuous type of weather that popularly stamps a season as being hot, cool, wet, dry, etc., but rather by the condition of the plants as determined by the interaction of all factors involved in growth. This may account for the seeming conflict of numerous and world-wide reports of powdery-mildew epidemics in the small grains in so-called hot, cool, wet, or dry seasons.

Environment may simultaneously influence immediate infection and conditioning of the plant to subsequent infection.

Rivera's reports that plants become susceptible to powdery mildew during periods of flaccidity or partial wilting of the host tissues were not confirmed. His results are attributed to the use of plants exposed to different environmental conditions before inoculation.

Knowledge of the greater susceptibility of tender plants and of the possible and doubtless frequent existence of a pseudo-resistance in susceptible varieties induced by environment should facilitate breeding for resistance and speed reduction of the present heavy loss in the affected areas.

PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

LITERATURE CITED

1. ASUYAMA, H. Important diseases of barley in Japan. 6 pp. 1946. (Mimeographed.)
2. ATKINS, I. M. Diseases of wheat and barley at Denton, Texas, April, 1947. U. S. Dept. Agr., Pl. Dis. Rptr. **31**: 242–243. 1947.
3. BRAUN, ALVIN J. Diseases of wheat and rye in northwestern Ohio. U. S. Dept. Agr., Pl. Dis. Rptr. **29**: 356–357. 1945.
4. BRETZ, T. W. Diseases on winter grains in Missouri. U. S. Dept. Agr., Pl. Dis. Rptr. **29**: 360–361. 1945.
5. ————. Diseases of small grains in Missouri. U. S. Dept. Agr., Pl. Dis. Rptr. **29**: 492–493. 1945.
6. BRODIE, HAROLD J. Further observations on the mechanism of germination of the conidia of various species of powdery mildew at low humidity. Canad. Jour. Res. (C) **23**: 198–211. 1945.
7. BUTLER, F. C. Diseases of barley. Agr. Gaz. N. S. W. **57**: 649–654. 1946.
8. CHEREWICK, W. J. Studies on the biology of Erysiphe graminis DC. Canad. Jour. Res. (C) **22**: 52–86. 1944.
9. CLINTON, G. P. Plant pest handbook for Connecticut. II. Diseases and injuries. Conn. Agr. Exp. Sta. Bul. 358. 1934.
10. CORNER, E. J. H. Observations on resistance to powdery mildews. New Phytologist **34**: 180–200. 1935.
11. DICKSON, JAMES G. Diseases of field crops. 429 pp. New York. 1947.
12. EWERT, R. Pflanzenkrankheiten und Witterung. Starkes Auftreten von Mehltau, Roggenbraunrost und Fritfliege im Herbst 1934. Mitt. Landw. Berl. **1**: 337. 1935.
13. FENNE, S. B. Loose smut and powdery mildew on wheat and barley very severe in Virginia. U. S. Dept. Agr., Pl. Dis. Rptr. **31**: 281. 1947.
14. FOISTER, C. E. The relation of weather to plant diseases. Conf. Empire Meteorologists, Agr. Sec. 50 pp. 1929.
15. FROMME, F. D. The culture of cereal rusts in the greenhouse. Torrey Bot. Club Bul. **40**: 501–521. 1913.
16. GRAF-MARIN, ALBERTO. Studies on powdery mildew of cereals. Cornell Agr. Exp. Sta. Memoir 157. 1934.
17. GRAINGER, JOHN. The ecology of Erysiphe graminis DC. Brit. Mycol. Soc. Trans. **31**: 54–65. 1947.
18. HAMMARLUND, C. Zur Genetik, Biologie und Physiologie einiger Erysiphaceen. Hereditas **6**: 1–126. 1925.
19. HEBERT, T. T., W. H. RANKIN, and G. K. MIDDLETON. Interaction of nitrogen fertilization and powdery mildew on yield of wheat. (Abstr.) Phytopath. **38**: 569–570. 1948.
20. HEY, G. L., and J. E. CARTER. The effect of ultra-violet light radiations on the vegetative growth of wheat seedlings and their infection by Erysiphe graminis. Phytopath. **21**: 695–699. 1931.
21. HONECKER, LUDWIG. Über die Modifizierbarkeit des Befalles und das Auftreten verschiedener physiologischer Formen beim Mehltau der Gerste, Erysiphe graminis hordei Marchal. Ztschr. Zücht. (A) **19**: 577–602. 1934.
22. ————. Über den derzeitigen Stand und die Aussichten der Bekämpfung des Mehltaubefalles der Gerste durch Züchtung. Prakt. Bl. f. Pflanzenbau. u. Pflanzenschutz **13** (12): 309–320. 1936.
23. ————. Mehltauschäden bei Getreide und ihre Bekämpfung. Mitt. Landw. Berl. **55**: 745–747. 1940.
24. ————. Resistenzzüchtung gegen Mehltau und Rost bei Gerste. Erfahrungen und Ergebnisse vierjähriger Züchtungsarbeit. Ztschr. Pflanzenzücht. **25**: 209–234. 1943.
25. JOHNSTON, C. O. Cereal diseases in Kansas in 1947. U. S. Dept. Agr., Pl. Dis. Rptr. **31**: 322–323. 1947.

26. ————————, HURLEY FELLOWS, and L. E. MELCHERS. Reaction of certain varieties of wheat to infections of powdery mildew at Manhattan, Kansas, 1932–1935. U. S. Dept. Agr., Pl. Dis. Rptr. **21**: 201–211. 1937.
27. LARSH, HOWARD M. Prevalence and destructiveness of cereal diseases in Arkansas during 1945. U. S. Dept. Agr., Pl. Dis. Rptr. **29**: 487–491. 1945.
28. LOWIG, E. Ueber den Einfluss der Kalisalze, insbesondere ihrer Anionen, sowie der Kieselsäure und des Stickstoffs auf die Mehltauresistenz von Getreide und Futterpflanzen. Landw. Jahrb. **81**: 273–335. 1935.
29. MACFARLAN, JOHN, and JOHN GRAINGER. Corn mildew on oats. Scot. Agr. **26**: 211–215. 1947.
30. MACKIE, J. R. Localization of resistance to powdery mildew in the barley plant. Phytopath. **18**: 901–910. 1928.
31. MIDDLETON, G. K. Importance of mildew in the soft winter wheat area. Tenth Eastern Soft Wheat Improvement Conference. Cincinnati, Ohio. pp. 12–13. Nov. 18–20, 1947.
32. MIX, A. J. Cereal diseases in Delaware and Maryland in 1945. U. S. Dept. Agr., Pl. Dis. Rptr. **29**: 583–586. 1945.
33. MONTEMARTINI, L. Note di fitopatologia. Riv. di Patol. Veg. **20**: 201–206. 1930.
34. MOORE, W. C. Cereal diseases in England and Wales. Ann. Appl. Biol. **31**: 360–362. 1944.
35. ————————. Cereal diseases. Their recognition and control. Min. Agr. and Fisheries [London] Bul. 129. 1945.
36. PADY, STUART M. Diseases observed on cereals in Kansas. U. S. Dept. Agr., Pl. Dis. Rptr. **29**: 527–530. 1945.
37. PRATT, ROBERTSON. Influence of temperature on the infection of wheat by the powdery mildew Erysiphe graminis tritici. Torrey Bot. Club Bul. **70**: 378–385. 1943.
38. ————————. Influence of light on the infection of wheat by the powdery mildew, Erysiphe graminis tritici. Torrey Bot. Club Bul. **71**: 134–143. 1944.
39. PRINCE, ALTON E. Small grain disease surveys in the Carolinas. U. S. Dept. Agr., Pl. Dis. Rptr. **29**: 632–643. 1945.
40. REED, GEORGE M. Influence of light on infection of certain hosts by powdery mildews. (Abstr.) Science, N. S. **39**: 294–295. 1914.
41. REID, D. A., and L. M. JOSEPHSON. Small grain diseases and Hessian fly in Kentucky in 1947. U. S. Dept. Agr., Pl. Dis. Rptr. **31**: 468–469. 1947.
42. ————————, and ————————. Small grain diseases in Kentucky in 1948. U. S. Dept. Agr., Pl. Dis. Rptr. **33**: 36–38. 1949.
43. RIVERA, V. Primo contributo allo studio della recettivita della Quercia per l'oidio. R. Accad. naz. dei Lincei, Rend. **22**: 168–173. 1913.
44. ————————. Ricerche sperimentali sulle cause predisponenti il frumento alla "Nebbia." Mem. d. R. stazione di patologia vegetale. 42 pp. 1915.
45. ————————. Cryptogamic epidemics and the environmental factors that determine them. Internatl. Rev. Sci. and Pract. Agr. [Rome] N. S. **2**: 604–610. 1924.
46. RIVERA, VINCENZO. Condizioni fisiologiche di predisposizione di tessuti vegetali ad attacchi crittogamici. Nuovo Gior. Bot. Ital. N. S. **39**: 689–690. 1932.
47. SEMPIO, C. Influenza della luce continua sulla resistenza del frumento all' Erysiphe graminis e meccanismo di tale resistanza. Riv. Biol. **35**: 137–145. 1943.
48. SPINKS, G. T. Factors affecting susceptibility to disease in plants. Jour. Agr. Sci. **5**: 231–247. 1913.
49. SUNESON, C. A. Powdery mildew on barley in California. U. S. Dept. Agr., Pl. Dis. Rptr. **23**: 137. 1939.
50. TAPKE, V. F. Barley powdery mildew (Erysiphe graminis hordei) influenced by environment previous to inoculation. (Abstr.) Phytopath. **38**: 25. 1948.
51. ————————. Further studies on barley powdery mildew (Erysiphe graminis hordei). (Abstr.) Phytopath. **39**: 505. 1949.
52. ————————. Flaccidity, turgidity, and predisposition in barley and wheat as factors in susceptibility to powdery mildew (Erysiphe graminis DC.). (Abstr.) Phytopath. **40**: 873–874. 1950.
53. TAYLOR, J. W., H. A. RODENHISER, and B. B. BAYLES. Physiologic races of Erysiphe graminis tritici in the Southeastern United States. Agron. Jour. **41**: 134–135. 1949.
54. TRELEASE, SAM F., and HELEN M. TRELEASE. Susceptibility of wheat to mildew as influenced by salt nutrition. Torrey Bot. Club Bul. **55**: 41–68. 1928.
55. ————————, and ————————. Susceptibility of wheat to mildew as influenced by carbohydrate supply. Torrey Bot. Club Bul. **56**: 65–92. 1929.
56. UBRIZSY, G. Adatok a Nyirseg lisztharmatgombainak (Erysiphaceae) ismeretehoz. Acta. Mycol. Hung. **3**(1–4): 28–33. 1946.

57. VOLK, A. Einflüsse des Bodens, der Luft und des Lichtes auf die Empfänglichkeit der Pflanzen für Krankheiten. Phytopath. Ztschr. 3: 1–88. 1931.
58. WESTON, W. A. R. Powdery mildew of cereals. Jour. Min. Agr. 52: 522–524. 1946.
59. YARWOOD, CECIL E. The tolerance of Erysiphe polygoni and certain other powdery mildews to low humidity. Phytopath. 26: 845–859. 1936.
60. ————————. The diurnal cycle of the powdery mildew Erysiphe polygoni. Jour. Agr. Res. [U. S.] 52: 645–657. 1936.

TRICHOTHECIUM FRUIT ROT OF GLASSHOUSE TOMATOES

ROBERT E. DEEMS[1]

(Accepted for publication February 23, 1951)

Specimens of an undescribed tomato fruit disease, previously observed and photographed by Dr. L. J. Alexander, were collected from five different glasshouses in Ohio from April through June, 1950. These glasshouses, four in Cleveland and its vicinity and one in Cincinnati, represented two widely separated sections of the State. The disease, which is of minor seriousness, is found first as a firm dark brown rot that usually develops into a soft watery black rot, its surface partially overspread with a pink-orange fungal growth. The diseased specimens were collected from three varieties of tomato (*Lycopersicon esculentum* Mill.): Ohio W-R Globe, Strain A Globe, and Marglobe. The causal organism was determined to be *Trichothecium roseum* Link.

This paper includes a description of the disease observed under natural and controlled environmental conditions, a record of inoculation experiments, and a description of the causal organism.

Ainsworth and Bisby (1) record that the type organism of *T. roseum* was described on apple by Link in Species Plantarum (1824). In succeeding years it was reported as parasitic on a wide variety of suscepts and saprophytic on various materials.

The fungus is most important economically on fruits. Workers have demonstrated that the bitter core rot of apples and pears, rots of bananas and oranges, and a rot following wounds on early cherries, apricots, and quinces are caused by *T. roseum* (3, 4, 17, 21). It has also been reported to cause a severe stem, leaf, and flower wet rot of glasshouse cucumbers and a fruit rot of honeydew melons and cantaloupes (13, 19, 30). In addition, it has been found on certain green timbers, oat seeds, maize ears, woolen fabrics, *Pteris longifolia* prothallia, opium poppy, soybean seeds, and hemp, and in the nodal tissue of maize plants (5, 6, 14, 16, 18, 23, 24, 26, 27, 28). It has also been found pathogenic upon humans (12). Furthermore, it has been isolated from cotton seeds, chestnuts, milk, cacao beans, coconut (copra), *Pinus sylvestris* seeds, and leather products (7, 8, 9, 10, 15, 27, 29).

OBSERVATIONS AND EXPERIMENTAL RESULTS

The first symptom of the disease on tomato fruits is a light brown lesion, generally occurring near the stylar end; this condition has been observed on fruits in all stages of development. Lesions typically radiate from the stylar end of the fruit, with invasion of new tissue often continuing until

[1] Graduate Fellow, Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, Wooster, Ohio.

The writer is indebted to Dr. L. J. Alexander, under whose direction this research was undertaken, for his stimulating and valuable advice and for his constant encouragement throughout the investigation.

the entire fruit is discolored (Fig. 1). *T. roseum* causes a firm dark brown rot, although usually in nature secondary organisms become quickly established so that a soft, watery, dark brown to black rot results. At advanced stages, varying according to environmental conditions, a discoid sporulation of light pink to orange-pink conidia appears. The orange-pink coloration and a powdery texture develop with age.

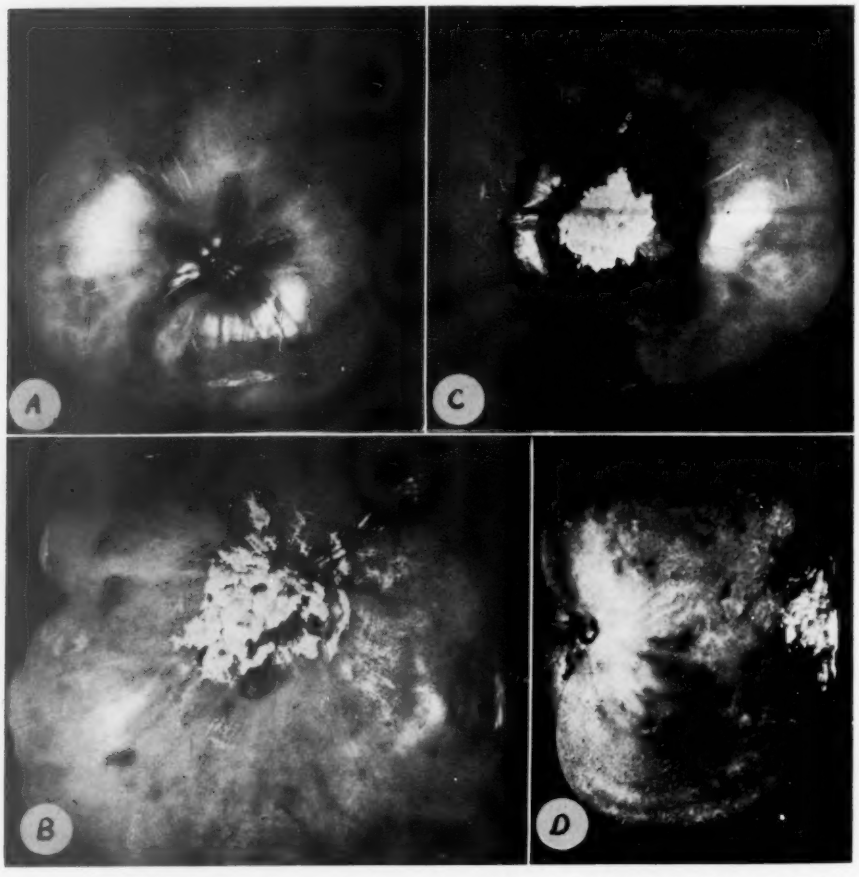

FIG. 1. Trichothecium fruit rot of tomato. Natural infection on Ohio W–R Globe (A) and Strain A Globe (B) varieties. Typical radiating young lesion in A, with blanched halo effect distal from the lesion; discoid, prostrate, powdery, orange-pink sporulation characteristic of advanced stages in B. Artificially induced rot on Ohio W–R Globe (C and D), with sporulation within the wound in the sectioned fruit.

Diseased fruits occur most commonly on the first three or four clusters of the glasshouse crop, with healthy fruits in the same cluster. No symptoms were observed upon the vegetative portions.

Tissue fragments from diseased fruits collected from the glasshouses listed in table 1 yielded isolates of *T. roseum*, the fungus being present in 123 of 156 tissue plantings. Conidia from each isolate were inoculated into green tomato fruits which had been previously sterilized by agitation for

5 min. in a 3.5 per cent solution of calcium hypochlorite. Each fruit skin was punctured four times through a droplet of conidia in sterile water; these inoculated fruits were kept in moist chambers at room temperature (25°–27° C.) and observed periodically. Characteristic symptoms were observable in each fruit after 14 to 21 days. The identical conidial suspension was atomized upon nonwounded tomato fruits but no disease symptoms resulted. A similar procedure with fruits *in situ* yielded similar results. Tissue isolations were made from each diseased fruit and only *T. roseum* was recovered. These isolates were then inoculated into sterilized green fruits and typical symptoms were produced.

Some variability in the fungal isolates from the various glasshouses was noted. Those isolates from fruits in Houses II, III, and V were slightly slower invaders and grew more upright than did the isolates from fruits in Houses I and IV. No other differences were observed. Spore arrangements and measurements were identical.

TABLE 1. -- Frequency of Trichothecium roseum among isolates obtained from 40 tissue fragments from 10 rotted tomato fruits in Ohio glasshouses, 1950

Source	Number of representative fruits used	Date collected	Number of tissue fragments		
			Cultured	Yielding T. roseum	Yielding other organisms
North Olmsted					
House I.....	2	Mar. 28	8	6	3
House II.....	1	Apr. 11	4	4	1
Vermilion					
House III	1	May 17	4	4	0
Cleveland					
House IV.....	3	Mar. 28	12	9	2
Cincinnati					
House V	3	May 11	12	8	3

Commonly only two secondary organisms were obtained from tissue fragments, a Gram-positive, white-pigmented and a Gram-negative, yellow-pigmented bacterium. Neither of these two organisms was parasitic on sterilized green tomato fruits inoculated by puncturing the skins, four times, through a suspension of bacteria in sterile water. The bacteria were reisolated only from that tissue destroyed as a result of needle punctures, thereby indicating their lack of pathogenicity. When these bacteria were inoculated into sterilized green tomato fruits in all combinations with *T. roseum*, symptoms similar to those induced by the fungus alone were produced, except that the rotted areas approached a soft watery dark brown to black. In each case, the fungus and the bacterium or bacteria introduced were recovered from tissue isolations.

The behavior of conidia on surfaces of uninjured fruit was unknown. Conidia suspended in sterile water and placed on potato-dextrose agar commenced to germinate after 6 hr. and approximately 90 per cent had germi-

nated within 24 hr. Similar conidia on sterile glass slides in moist chambers scarcely germinated: after 48 hr. only 1–2 per cent had germinated and germ tubes grew very little. The conidial suspension also was atomized on the disinfected surfaces of tomato fruits. Some of the glasshouse fruits were enclosed in nitrocellulose moist chambers, others remained in the open. Fruits in the laboratory, likewise, were either placed in moist chambers or left in the open. After 48 hr. portions of the tomato skins were stripped from the fruits and stained with lacto-phenol cotton blue to facilitate observation of conidial germination. Very few conidia were found on fruits left in the open and none had germinated. On fruits in the moist chambers not more than 1–2 per cent of the conidia had germinated, and there was no evidence of direct penetration or of any special penetration organs.

The question arose as to when primary invasion occurred under glasshouse conditions. To investigate the possibility of blossom infection, a spore suspension, 90 per cent viable, was atomized upon 30 blossoms just following anthesis, during the first week of April, 1950. Fifteen comparable blossoms sprayed with sterile water developed, in all but two cases, into small fruits. Twenty-three of those blossoms atomized with the conidial suspension later turned brown and dropped. Eighteen of these blighted blossoms were tested for the presence of *T. roseum;* the corolla was removed and the remaining portion of the blossom was placed on potato-dextrose agar in a Petri dish. *T. roseum* was isolated from each blossom. Those blossoms remaining apparently healthy were tested in a similar manner but *T. roseum* was not present. In similar experiments at a later date, when temperatures were appreciably higher, no blight symptoms occurred.

The most virulent isolate of *T. roseum*, previously passed twice through tomato fruits, was inoculated by the puncture method into apples, bananas, oranges, peaches, pears, and quinces. A firm brown rot was produced in each case.

THE CAUSAL ORGANISM

The causal organism was identified as *T. roseum* Link. As far as the author is aware this is the first report of *T. roseum* being pathogenic on tomato, although Plowright (22), in 1881, reported a rare fruit disease peculiar to tomatoes grown under glass which was similar to this disease in several respects. He classified the pathogen as *Dactylium lycopersici* (Saccardo (25) listed the organism *Cephalothecium lycopersici* (Plowr.) Sacc.), and stated that the disease "commences upon the base of the fruit near the attachment of the stalk." Furthermore, his observations and illustrations showed three conidia borne upon conidiophores slightly bulbous at the apex. These statements are in opposition to the present work but his further description, limited as it is, might very well place the causal organism in synonymy with *T. roseum*.

On potato-dextrose agar the hyphal filaments of new growth are irregu-

larly septate with septa forming with age, until at maturity septation is quite regular. Colonies are first thin, white, and radiating, later pale pink, and still later the mature portions are orange-pink. Growth is floccose, prostrate, and limited. Aerial growth consists almost entirely of conidia and conidiophores. Conidiophores are upright, simple, and occasionally septate with orange-pink, bicelled (occasionally unicelled), smooth conidia borne acrogenously (Figs. 2, 3). The conidia are variable in conformation, being primarily piriform or oblong-ovate. Conidial measurements were 11.4–21.1 by 5.2–11.9 microns; the basal cell of the conidium is truncate to pointed at the basal attachment and is 5.7–12.3 microns long as compared to 5.3–10.6 microns for the terminal cell.

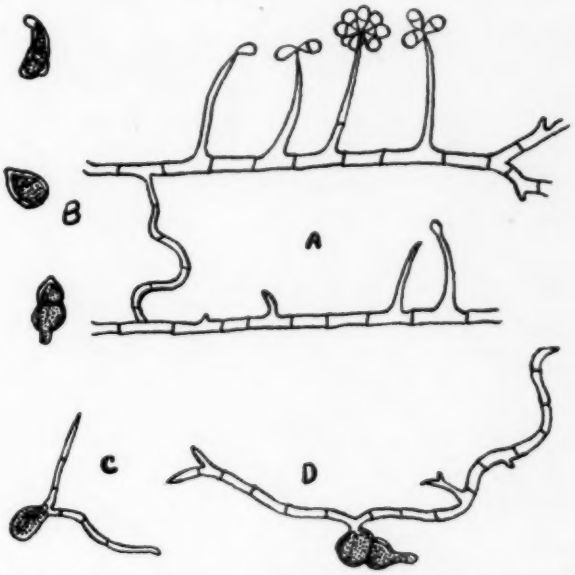

FIG. 2. Camera lucida reconstruction of morphological characteristics of *Trichothecium roseum*. A, successive sporulation stages with conidia borne acrogenously, one to many per head (approx. × 390). Upright conidiophores are usually aseptate, occasionally septate. B–D, unicellular and bicellular conidia (approx. × 850) germinating on potato-dextrose agar: B, 6 hr.; C, 12 hr.; D, 24 hr.

LeClerg (20) states that the cultural data obtained from *T. roseum* are the same upon Czapek, potato, peptone, maltose, inulin, levulose, and dextrose agars. The described organism complies in reaction upon potato-dextrose, Czapek, and maltose agars and is considered similar. The reader is referred to this work and to a more complete cultural study by D'Oliveira (11).

DISCUSSION

The 1950 spring glasshouse crop of Ohio tomatoes was grown under adverse weather conditions. Sunlight was limited and temperatures were relatively low. In those houses where the disease was observed, plants were

either pollinated by means of an electric vibrator, or their fruits were "set" with hormones; in either case injury to the young fruits or to neighboring fruits would readily occur. Since *T. roseum* grows saprophytically on organic substrates and is reportedly widespread, inoculum would probably be present in the glasshouse atmosphere throughout the early portion of the growing season. Conditions would therefore be near optimum for invasion by the pathogen.

The critical temperature range of *T. roseum* has been studied intensively by Adeline Ames (2). She found that the optimum for germination was 19°–20° C., and that conidia were killed at 36° C. In addition, 20°–22° C. was maximum for growth and fruiting, with small white colonies, and no fruiting bodies developed at 30° C. These temperature data help to explain

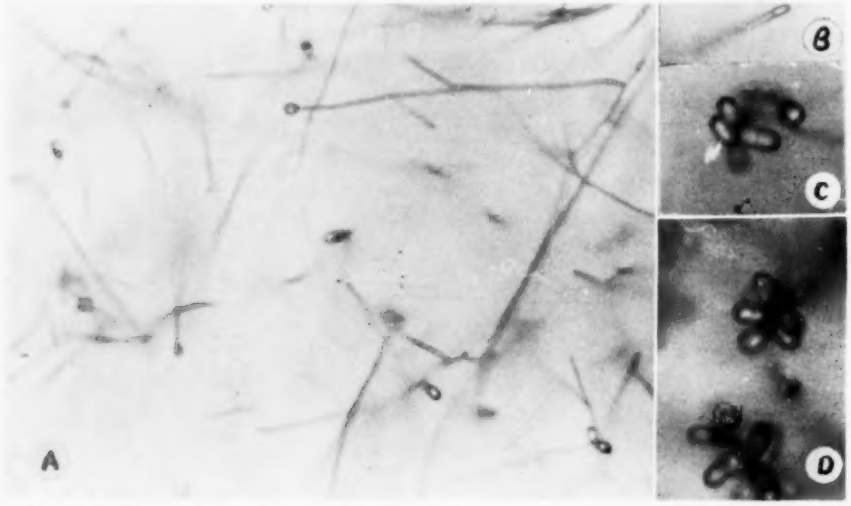

FIG. 3. Photomicrographs of *Trichothecium roseum*. A, conidiophores with one and two conidia (approx. × 150). B, conidium forming (approx. × 750). C and D, conidial heads with numerous mature bicellular conidia (approx. × 750).

the lack of development of the disease with the advent of warm weather. Possibly this is the reason experiments concerning the blossom-blight aspect of the disease gave variable results, since temperatures in the experimental glasshouse during the second trials were generally 30° C. or above.

The available evidence indicates that the disease will not become economically important on glasshouse tomatoes unless a particularly cool moist growing season occurs. However, it could prove damaging to tomatoes stored under cool, relatively moist conditions.

SUMMARY

A minor fruit-rot disease of *Lycopersicon esculentum* Mill. is caused by *T. roseum* Link. In nature, symptoms caused by *T. roseum* are generally supplemented by those caused by secondary saprophytes. The disease is illustrated and described under natural and controlled environmental con-

ditions. Primary invasion occurs only following fruit injury, in the light of the evidence presented in this paper. Experimental results also indicate that a blossom-blight disease of tomatoes can be caused by *T. roseum* under favorable environmental conditions. Inoculation tests on apple, banana, orange, peach, pear, and quince demonstrate that this fungus is similar to previously reported strains on other suscepts.

OHIO AGRICULTURAL EXPERIMENT STATION
WOOSTER, OHIO

LITERATURE CITED

1. AINSWORTH, G. C., and G. R. BISBY. A Dictionary of the Fungi. Mycological Institute, Kew, Surrey. 2nd ed. 431 pp. 1945.
2. AMES, A. The temperature relations of some fungi causing storage rots. Phytopath. **5**: 11–19. 1915.
3. BECZE, G. V. Beitrage zu den wahrend des Transportes und wahrend der Reifung auftretenden Faulniskrankheiten der Bananen. Zentralbl. für Bakt. II, **86**: 381. 1932. (Abstr. in Rev. Appl. Myc. **12**: 39. 1933.)
4. BROOKS, C., D. F. FISHER, and J. S. COOLEY. Apple rots. Phytopath. **4**: 403. 1914.
5. BURGESS, R. A contribution to the study of the microbiology of wool. Jour. Textile Inst. **19**: T315–T322. 1928. (Abstr. in Rev. Appl. Myc. **8**: 38. 1929.)
6. CIFERRI, R. Informe de fitopatologia. Principales enfermedades de las plantas cultivadas, observadas en el curso del ano 1926. Segundo Informe Anual Estac. Nac. Agron. Moca, Republica Dominicana, 1926. pp. 36–44. 1927. (Abstr. in Rev. Appl. Myc. **7**: 20–22. 1928.)
7. — ————. Studies on cacao. Jour. Dept. Agr., Puerto Rico, **15**: 223–286. 1931. (Abstr. in Rev. Appl. Myc. **11**: 30–31. 1932.)
8. COLIN-RUSS, A. A contribution to the study and control of mould growth in leather and other material. Jour. Intern. Soc. Leath. Chem. **24**: 395–408. 1940. (Abstr. in Rev. Appl. Myc. **20**: 304. 1941.)
9. CRAWFORD, R. F. Fungi isolated from the interior of cotton seeds. Phytopath. **13**: 501–503. 1923.
10. CUMMINS, H. A., V. C. E. KENNELLY, and M. GRIMES. A study of fungi found in milk. Scient. Proc. Roy. Dublin Soc. **19**: 311–319. 1929. (Abstr. in Rev. Appl. Myc. **9**: 185. 1930.)
11. D'OLIVEIRA, B. Podridao rosea do coracao das Macas. Revista Agronomica **18**: 3, 49–63; 4, 9–67. 1930. (Abstr. in Rev. Appl. Myc. **10**: 320. 1931.)
12. DOSA, A. Über das Trichothecium roseum. Derm. Wschr. **104**: 548–549. 1937. (Abstr. in Rev. Appl. Myc. **16**: 674. 1937.)
13. FISHER, G. W. A study of the fruit diseases occurring in a Midwestern market. Butler Univ. Bot. Studies, Indianapolis, Ind. **1**: 105–127. 1930.
14. FULLER, W. H., and A. G. NORMAN. Biochemical changes involved in the decomposition of Hemp bark by pure cultures of fungi. Jour. Bact. **1**: 667–671. 1945.
15. BARBOWSKI, L. Przyczynek do zmajomosci mikroflory grzybney masion drzew lesnych. [Contribution to the knowledge of the fungal microflora of forest tree seeds.] Prace Wydz. Chor. Rosl. panstw. Inst. Nauk. Gosp. wiejsk Bydgoszczy **15**: 5–30. 1936. (Abstr. in Rev. Appl. Myc. **16**: 147. 1937.)
16. GENTNER, G. Schadigung des Haferkorns durch Mikroorganismen und die fritfliege. Prakt. Blatter Pflanzenblau und Pflanzenschutz, **3**: 6–9. 1925. (Abstr. in Rev. Appl. Myc. **4**: 536. 1925.)
17. HOPKINS, J. C. Report of plant pathologist for year ending December 31, 1929. Rept. of the Secretary, Dept. of Agr., Southern Rhodesia, for the year 1929, pp. 84–86. 1930. (Abstr. in Rev. Appl. Myc. **9**: 627. 1930.)
18. HOWARD, N. O. The control of sap-stain, mold, and incipient decay in green wood with special reference to vehicle stock. U. S. Dept. Agr., Bul. 1037. 1922.
19. KALASHNIKOFF, K. J. Trichothecium roseum Link. on cucumber plants under glass. Pl. Prot. Leningr. 1935, **7**: 136–139. 1935. (Abstr. in Rev. Appl. Myc. **15**: 699. 1936.)
20. LECLERG, E. L. Cultural studies of some soil fungi. Mycologia **22**: 186–213. 1930.
21. PEYRONEL, B. Il marciume amaro o marciume del cuore delle mele e delle pere. Boll. mensile della R. Staz. di Patologia vegetale. **2**: 23–27. 1921. (Abstr. in Rev. Appl. Myc. **1**: 25. 1922.)
22. PLOWRIGHT, C. B. On the fungoid diseases of the tomato. The Gardeners Chronicle, Nov. 12, 1881.

23. PORTER, C. L. A study of the fungous flora of the nodal tissues of the corn plant. Phytopath. **17**: 563–568. 1927.
24. REINMUTH, E. Die parasitare Blattdurre eine fur den Mohnbau bemerkenswerte Krankheit. (Parasitic leaf desiccation, a noteworthy disease in poppy cultivation.) Angew. Bot. **24**: 273–277. 1942. (Abstr. in Rev. Appl. Myc. **22**: 39. 1943.)
25. SACCARDO, P. A. Sylloge Fungorum **10**: 549. 1882.
26. TERVET, I. W. The influence of fungi on storage, on seed viability and seedling vigor of soybeans. Phytopath. **35**: 3–15. 1945.
27. THOMPSON, A. Division of Mycology, Annual Report for 1931. Dept. Agr. Straits Settlements and Fed. Malay States (Technical Reports for the year 1931). Bull. 12, Gen. Ser. pp. 48–52. 1933. (Abstr. in Rev. Appl. Myc. **13**: 215. 1934.)
28. VAN HOOK, J. M. Indiana fungi, XIII. Proceed. Ind. Acad. Sci. **44**: 55–64. 1935. (Abstr. in Rev. Appl. Myc. **15**: 283. 1936.)
29. VOGLINO, P. Il servizio di controllo fitopatologico sulle Castagne destinate agli Stati Uniti d'America nella campagne 1928 esercitato dal R. Osservatorio di Fitopatologia di Torino. Nuovi Ann. Agr. **8**: 319–344. 1928. (Abstr. in Rev. Appl. Myc. **8**: 614. 1929.)
30. WIANT, J. S. Investigations of the market diseases of cantaloupes and honey dew and honey ball melons. U. S. Dept. Agr., Tech. Bul. 573. 1937.

TWO CANKERS OF CINCHONA IN GUATEMALA CAUSED BY PHYTOPHTHORA CINNAMOMI AND P. PARASITICA[1]

ELLIS F. DARLEY AND MARCO A. FLORES[2]

(Accepted for publication March 22, 1951)

INTRODUCTION

Two distinct bark diseases of cinchona, called stripe canker and girdle canker, have caused serious losses in some plantings in Guatemala. Practically all the plantings consist of clones that are selections of *Cinchona pubescens* Vahl (*C. succirubra* Pavon), the Ledger form of *C. officinalis* L., or, more usually, hybrids of the two grafted on rootstocks of *C. pubescens*. Stripe canker, shown in the present investigation to be caused by *Phytophthora cinnamomi* Rands, may be found in the rootstock and scion of practically all the clones. Girdle canker, shown to be caused by *P. parasitica* Dast., appears to be localized just above the union in the scion of the Ledger selections and hybrids. Although these two diseases have been recognized for the past three or four years, their cause has not been definitely proved.

In 1886 Eekhout (7) described a bark canker of cinchona from the East Indies, which is probably the same disease now known as stripe canker. Coster (2, 3) reported an undesignated species of *Phytophthora* as the cause in West Java.

Thompson (10) first reported *P. cinnamomi* on cinchona in Malaya in 1939. He isolated the fungus from decaying tissue in the main root system. In some trees he observed also a decaying and splitting of the bark just above and below the soil level, but he was not able to isolate the fungus from the split bark. In inoculation studies, the fungus developed slowly and the typical symptoms of the decay and splitting of the bark were not reproduced. He concluded that his strain of *P. cinnamomi* was a weak parasite on cinchona.

In 1947 Crandall (4) reported *P. cinnamomi* as the cause of a nursery root rot of cinchona in Peru. Only the smaller roots were affected, the larger roots and crown remaining healthy. No symptoms of stripe canker were reported.

[1] A contribution from the Instituto Agropecuario Nacional, a technical agricultural service organization for Guatemala, operated jointly by the Government of Guatemala and by the Office of Foreign Agricultural Relations, U. S. Department of Agriculture. United States participation in this work was carried out as part of the Point IV program in Guatemala, administered by the Technical Cooperation Administration, U. S. Department of State.

[2] Formerly Agriculturist (Pathology) and Head, Department of Plant Pathology, and Assistant Pathologist, respectively, Instituto Agropecuario Nacional, an agricultural experiment station operated jointly by the Guatemalan Ministry of Agriculture and the U. S. Department of Agriculture.

The authors gratefully acknowledge the assistance given by John and Owen Smith of Fincas Moca and Panama, and of the Merck Co., through B. A. Krukoff, Ralph Pinkus, and L. R. Holdridge of Finca El Naranjo, for use of facilities and plant material on their plantations.

Crandall and Davis (5), in their survey of cinchona root rot in Latin America in 1944, described from Guatemala two types of lesions that may have been a combination of stripe and girdle canker. Very narrow streak lesions extended from infected roots up through the union and into the stem of the scion and connected with a larger girdling lesion on the scion. A year later Reinking (9) described more clearly what is probably the girdle canker as it is now recognized. In grafts of the Ledger form of *Cinchona officinalis* on *C. pubescens* he noted browned and dead bark on the Ledger scion where it joined the rootstock. A species of *Nectria* was found fruiting on the dead bark, but he considered this as secondary and suggested that a *Phytophthora* might be responsible for the trouble. In the same year Crandall and Davis (6) reported from Peru a wilt and stem canker of the terminal branches of cinchona caused by *P. parasitica*. Infection originated either in the petiole region of the new leaves or in the succulent stem tissue. Girdle of the main stem was not listed as a symptom. They reported that the same disease was present in Guatemala, although it was not seen by the authors in 1948 or 1949. What appears to be the same disease, however, is very serious in Costa Rica, where it is referred to as top blight, and from which *P. parasitica* has been isolated by C. M. Tucker.[3]

THE DISEASES

Stripe canker. Infection apparently originates in the roots and advances upward through the union and into the stem of the tree. The disease is characterized by vertical, linear, slightly sunken stripes of dead and dying inner bark (Fig. 1). The stripes may vary in width from $\frac{1}{4}$ in. to more than 1 in., and in length from a few inches above the ground in young lesions to the full length of the tree as the disease progresses. There may be several infection courts on the roots, giving rise to as many stripes on the stem (Fig. 1, C). In some instances multiple stripes develop on the stem from a single infection on the roots because of a "branching" of the lesion. As the disease spreads upward, droplets of dark, sticky exudate appear on the surface of newly infected bark. These symptoms are practically indistinguishable from those described and illustrated by Rands (8) in his work on the stripe canker of cinnamon in which he described the fungus, *Phytophthora cinnamomi*.

The leaves of infected trees of some clones become yellowed when several stripes develop, and the trees may eventually die. Trees of other clones, however, may have many stripes without apparent injury to the leaves or general health of the tree.

Apparently stripe canker is soil-borne. It is a common observation in Guatemala that infection spreads from tree to tree in a downhill direction, probably from inoculum carried on and in the soil by rain. As will be

[3] From correspondence in the files of the Experimental Plantations Co., Finca El Naranjo, Guatemala, furnished to the writers through the courtesy of B. A. Krukoff, Manager.

FIG. 1. Symptoms of stripe canker on cinchona: A–C, natural infection; A, long, narrow, sunken stripes of diseased bark; B, same as A with outer bark removed to show dead and discolored tissues of inner bark (note stripes coming up from roots); C, root of infected tree, with outer bark cut away to show path of multiple infection through root into stem. D and E, tree inoculated with *Phytophthora cinnamomi;* D, point of inoculation about 4 ft. from ground showing bandage covering inoculation, with stripe faintly visible above and below the bandage as bark has become slightly sunken: E, same as D with outer bark cut away to show dead and discolored tissue of stripe.

pointed out later, the fungus was isolated from the soil in the vicinity of infected trees.

Girdle canker. Girdle canker, as its name implies, girdles the tree. The bark of the scion above the graft union is killed and becomes sunken in a horizontal band measuring from two to several inches in width (Fig. 2). The sunken appearance is accentuated by the inrolling of callus tissue above and below the dead area. The canker may spread up the stem of the scion, but in no tree was infection found to go below the graft union and into the rootstock more than ¼ in. Leaves turn yellow and there is a gradual die-back of the twigs and branches, culminating eventually in the death of the entire tree. Previous observations by one of the plantation managers indicated that infection took place through the leaves and petioles of small

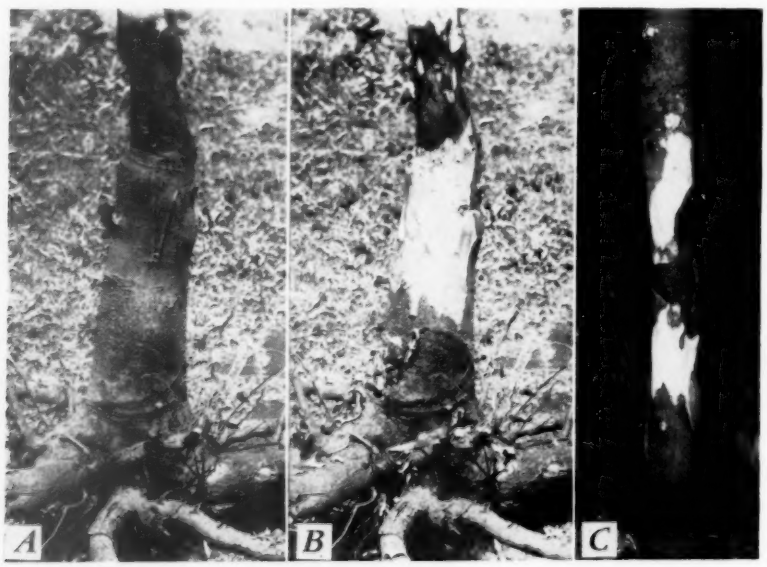

FIG. 2. Symptoms of girdle canker on cinchona: A and B, naturally infected tree completely girdled just above graft union; girdle appears accentuated because of inrolling of callus above and below diseased tissue; outer bark removed in B to show healthy tissue above and below girdle. C, tree inoculated with *Phytophthora parasitica* completely girdled in about 3 months; outer bark cut away shows extent of dead tissues of inner bark.

shoots at the base of the scion and advanced into the bark of the main stem where girdling occurred. Many instances were found by the authors where the dead shoots were still attached to the scion at the site of the girdle lesion. Other cases were found where the leaves and petioles of these small shoots were beginning to wilt and die and *P. parasitica* was isolated from such shoots.

Typical girdling was not found on small seedlings or grafts in nursery beds. There were, however, trees in nurseries on which the terminal leaves and stem were wilted and dead. As will be pointed out later, *P. parasitica* was isolated from these nursery trees. In these respects the girdle canker and infection in the nursery are similar to the disease described by Crandall

and Davis (6) in Peru and to the top blight in Costa Rica. It is not known whether the Peruvian or Costa Rican isolates of *P. parasitica* cause girdle canker.

ISOLATION OF CAUSAL ORGANISMS

Isolations were first made by C. M. Tucker early in 1948 from material sent to him by the Merck Co. from their plantations in Guatemala and Costa Rica. He obtained *P. cinnamomi* and *P. parasitica* from stripe canker and girdle canker lesions, respectively.[4] Additional isolations made by Tucker gave the same results.

In late 1948, the authors made isolations from stripe and girdle canker lesions and from the soil in an area in which the trees were badly diseased with stripe canker. In every case where isolations were successful, *P. cinnamomi* was obtained from stripe-canker lesions and *P. parasitica* from girdle canker. Isolations from diseased nursery stock yielded both fungi, *P. cinnamomi* being obtained from stripe-cankered trees and *P. parasitica* from plants whose top leaves and stems were wilted and dead. The latter plants had not developed typical cankers. The manner in which they were being attacked would prevent such a canker from forming as the plants were being killed progressively from the tip downward. As mentioned above, *P. parasitica* was also isolated from the wilted leaves of small shoots at the base of larger trees in the field.

Using Tucker's apple technique for isolating *Phytophthora* from diseased tissues (11), isolations were made from soil taken at a depth of 3 to 6 in. in the vicinity of stripe-cankered trees. *P. cinnamomi* was obtained in several cases. A similar method has since been described by Campbell (1) in relation to the little-leaf disease of pines in the United States.

PATHOGENICITY STUDIES

Preliminary inoculations were first made with both species of fungi on seedling trees of *C. pubescens* and with *P. parasitica* on seedlings of the Ledger form of *C. officinalis* growing in Guatemala City. In 21 days stripe cankers 3 to 6 in. long had developed on trees of *C. pubescens* inoculated with *P. cinnamomi*. On trees of *C. pubescens* inoculated with *P. parasitica* there was no evidence of positive attack by the fungi except for a slight discoloration of the tissues immediately surrounding the point of inoculation, a condition that confirms the observation reported above—that in natural infection of girdle canker the disease does not spread downward into the rootstock of *C. pubescens*. The Ledger seedlings inoculated with *P. parasitica* were killed, but no localized girdle lesions were formed, probably because the trees were so small.

On the basis of these inoculations, one isolate each of *P. cinnamomi* and *P. parasitica* was selected for extensive inoculations on various clones in field plantings. Five trees of each of 19 clones were inoculated with each isolate. Inoculum was grown for about 7 days on sterilized corn kernels

[4] See footnote 3.

partially submerged in potato-dextrose agar in Petri plates. At the time of inoculation a small wound was made in the bark of the tree with the point of a knife and one kernel of corn inserted in the wound. The wound was then covered with a small pad of cotton. The cotton was covered with a slightly larger piece of cellophane, which was tied to the tree (Fig. 1, D). Sterile corn kernels were inserted in similar wounds in control trees. Inoculations with *P. parasitica* were made about 12 in. above the graft union. In earlier experiments, inoculations with *P. cinnamomi* were made about 4 ft. above the ground (Fig. 1, D), and later in the rootstock just below the ground line. There was no difference in the results due to position of the infection court.

Definite stripes began to appear on trees inoculated with *P. cinnamomi* in about 12 days. After 30 days trees of all clones had developed stripes that varied from 8 to 16 in. in length. By the end of 2 months some of the stripes were 72 in. long and by 4½ months were more than 10 ft. long (Fig. 1,E). Typical sinking of the bark was evident after 60 days, as were also the droplets of dark exudate from the bark most recently invaded. *P. cinnamomi* was reisolated from some of these trees. On one clone, designated Z-15, stripes developed as rapidly as on other clones for the first 30 days. After that they ceased to elongate, indicating that the clone had some resistance to the disease. It is a common observation among the cinchona growers that Z-15 is one of the more vigorous clones and more tolerant of adverse conditions. In some naturally infected trees, stripes may develop the full length of the stem. In others, stripes 2 to 5 in. long at the base of the trees were no longer in June 1949 than when first noted in October 1948. These differences indicate that strains of the fungus exist.

On 15 clones, one of which was a selection of the Ledger form of *C. officinalis*, there was only slight infection from inoculation with *P. parasitica*. In many cases death of the bark tissue extended less than 1 in. from the point of inoculation, and after 130 days the infection appeared to have been walled off by callus tissue. These trees appeared to be very resistant. On four clones, however, three of which were selections of Ledger and one a hybrid, the lesions developed rapidly and increased in width until within 3 months the trunks of the trees had been girdled completely (Fig. 2, C). The fungus was reisolated from these trees. These experimental results were not in harmony with field observations. Inoculated trees of clone Z-92 were classed as resistant, whereas in the field some plantings of the clone had been practically eliminated as a result of girdle canker. This discrepancy would indicate that here again there are different strains of *P. parasitica* that cause girdle canker.

SUMMARY

Stripe canker and girdle canker, two distinct bark diseases of cinchona, hitherto undescribed in the Western Hemisphere, have caused serious losses

in some plantations in Guatemala. Isolation and inoculation studies showed that the diseases are caused by *Phytophthora cinnamomi* and *P. parasitica*, respectively.

P. cinnamomi is soil-borne and attacks all the clones tested. Only one, clone Z-15, appeared to have any degree of resistance. The girdle-canker fungus apparently enters the host through small succulent shoots near the base of the tree. Many of the clones inoculated were highly resistant to this disease.

Differences noted between results of experimental inoculations and the reactions of various clones in the field indicate that there are probably physiological strains of each fungus involved in these two diseases in Guatemala.

INSTITUTO AGROPECUARIO NACIONAL
GUATEMALA CITY, GUATEMALA

LITERATURE CITED

1. CAMPBELL, W. A. A method of isolating Phytophthora cinnamomi directly from the soil. U. S. Dept. Agr., Pl. Dis. Reptr. **33**: 134–135. 1949.
2. COSTER, CH. Het werk van het Proefstation West-Java in 1940. Bergcultures **15**: 1124–1133. 1941.
3. ———. The work of the West Java Research Institute, Buitenzorg, 1938–1941. Empire Jour. Exp. Agr. **10**: 22–30. 1942.
4. CRANDALL, BOWEN S. Cinchona root disease caused by Phytophthora cinnamomi. Phytopath. **37**: 928–929. 1947.
5. ———, and WILLIAM C. DAVIS. Occurrence of cinchona root rots in the Americas. U. S. Dept. Agr., Pl. Dis. Reptr. **28**: 926–929. 1944.
6. ———, and ———. Phytophthora wilt and stem canker of cinchona. Phytopath. **35**: 138–140. 1945.
7. EEKHOUT, G. W. De heer kessler over kina. Tijdschr. v. Nijv. Landb. Nederland.-Indië **33**: 539–542. 1886.
8. RANDS, R. D. Streepkanker van kaneel, veroorzaakt door Phytophthora cinnamomi n. sp. Buitenzorg Inst. v. Plantenziekten Meded. 54. 53 pp., illus. 1922.
9. REINKING, O. A. Report on cinchona diseases in Guatemala. U. S. Dept. Agr., Pl. Dis. Reptr. **29**: 432–439. 1945.
10. THOMPSON, A. Notes on plant diseases in 1939. Malayan Agr. Jour. **28**: 400–407. 1940.
11. TUCKER, C. M. Taxonomy of the genus Phytophthora de Bary. Mo. Agr. Exp. Sta. Res. Bul. 153. 1931.

A VIRUS RING SPOT OF ODONTOGLOSSUM ORCHID: SYMPTOMS, TRANSMISSION, AND ELECTRON MICROSCOPY

D. D. Jensen and A. Herbert Gold

(Accepted for publication April 4, 1951)

The experimental transmission of viruses infecting orchids has been previously reported for only four genera, *Dendrobium*, *Cattleya*, *Cymbidium*, and *Vanda*. Nobrega (7) in 1947 transmitted a virus from *Dendrobium nobile*, by mechanical inoculation, to *Nicotiana tabacum*, *N. rustica*, and *N. glutinosa* in Brazil. However, he failed to infect healthy *Dendrobium*, *Cattleya*, or *Oncidium*. In 1949 Jensen (3) reported transmission of a flower breaking virus from *Cattleya* to *Cattleya* in California by means of the green peach aphid, *Myzus persicae* (Sulzer). A widespread virus disease of *Cymbidium* was found by the same writer (4, 5) to be mechanically transmissible. The first purification of an orchid virus was reported in 1950 and 1951 by Gold (1, 2), who described and illustrated the sinuous rod-shaped virus particles isolated from a mosaic Cymbidium plant. A virus causing color breaking in the flowers of *Vanda* Miss Joaquim in Hawaii was reported by Murakishi (6) in 1950 to be transmissible by grafting and by juice inoculation, but not by two species of aphids tested.

The present paper reports transmission and purification of a ring-spot virus of *Odontoglossum grande* Lindl., the symptoms of which are markedly different from those described for other known orchid viruses. To the knowledge of the authors, this is the first record of a virus disease in Odontoglossum orchids.

In November 1949 a plant of *Odontoglossum grande* was received which had symptoms apparently caused by a virus. The plant was large, but was making little growth. The shriveled pseudobulbs carried three normal-sized leaves, two of which had concentric necrotic rings (Fig. 1, A). This plant served as the original source of virus for these investigations.

TRANSMISSION OF RING-SPOT VIRUS

The healthy plants of *Odontoglossum grande*[1] used in these tests were in a vigorous condition and had four to seven pseudobulbs. All test plants used in the host-range study were grown from seed in the greenhouse. For inoculation, the leaves of the test plants were sprinkled lightly with carborundum dust and then rubbed with a cotton swab soaked in infective juice. Within 5 min. after inoculation, these leaves were washed with tap water. A control plant was maintained for each test plant inoculated; all plants were kept together on the same bench in the greenhouse, and all controls have remained healthy.

Four of the control plants received the following treatment to deter-

[1] The writers acknowledge their gratitude to the E. W. McLellan Company of San Francisco for donating the healthy *Odontoglossum grande* orchids used in this study.

mine whether necrotic lesions could be produced in the absence of ring-spot virus. All leaves were sprinkled with carborundum. Using a cotton swab, the right half of each leaf was then rubbed with juice from a healthy plant and the left half with distilled water. No lesions developed on any leaves.

Five healthy plants were inoculated with freshly expressed juice from the original diseased plants. All five developed symptoms and subsequently served as inoculum for four additional tests. The four subinoculated plants also developed symptoms. The time between inoculation of test plants and appearance of the first symptoms was from 10 to 40 days, averaging 21 days. The incubation period in days for the individual plants follows: 10, 11, 11, 11, 14, 21, 31, 40, 40.

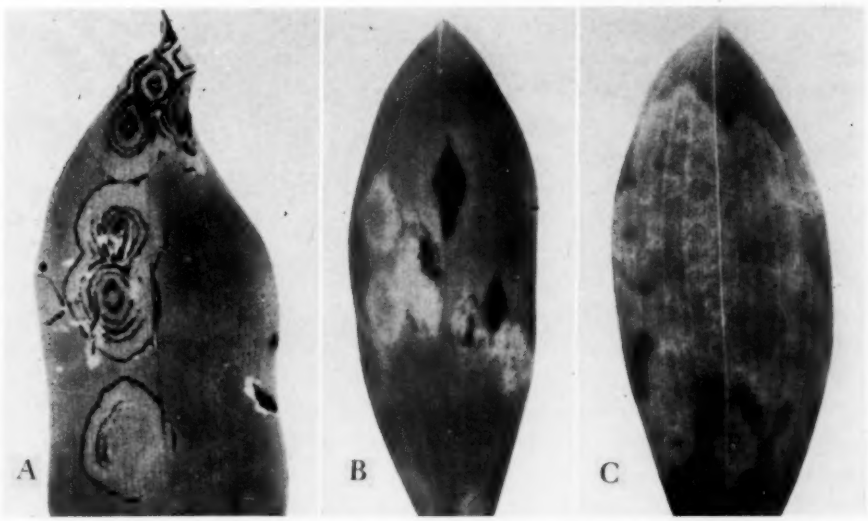

FIG. 1. Leaves of *Odontoglossum grande* with symptoms of ring-spot virus. A. Concentric necrotic rings on leaf from naturally infected plant. B, C. Leaves from experimentally infected plants photographed 43 days after infection: B, diamond-shaped areas of light green, yellow, and necrotic tissue; C, light green rings and spots coalescing and overlapping.

A single healthy plant was inoculated with an aliquot of the purified virus preparation which had been used for electron microscopy. This preparation was suspended in distilled water and used as inoculum 2 days after the juice had been extracted from the leaves of diseased plants. The first symptoms of the disease appeared on the leaves as necrotic spots 10 days after inoculation.

SYMPTOMS OF RING-SPOT VIRUS ON ODONTOGLOSSUM GRANDE

Leaf symptoms caused by ring-spot virus vary considerably among infected plants. Typically, the first evidence of disease appears as small necrotic spots or rings on the upper surface of one or more of the older inoculated leaves or as light green to pale yellow, circular, oval, or diamond-shaped areas, usually on somewhat younger leaves. These spots may be distinct or may present only a mild halo effect (Fig. 1, C). They vary

from 0.5 to 2 cm. in diameter. Within a few days after the first chlorotic symptoms appear, the margins of the affected areas become yellow, the enclosed tissue frequently remaining light green. Within a few weeks after first symptoms, the outline of some rings may be partially or entirely necrotic, the middle portion remaining light green or yellow. In some cases the entire zone becomes necrotic above (Fig. 1, B) and the under surface appears water-soaked.

The necrotic rings are usually single, but there may be several concentric brown to black rings with green to yellow tissue between the necrotic lines (Fig. 1, A) and the central spot necrotic or normal. The ring spots may be isolated from each other by normal tissue or may overlap or coalesce to form larger compound patterns.

The first leaves to show symptoms are usually more severely affected than later leaves. Some affected leaves turn yellow and drop within 2 to 3 months. Others with ring symptoms may persist for several months.

Three Odontoglossum plants were inoculated on only one half of each leaf to determine how soon symptoms appeared in the noninoculated portions of the leaves. The first symptoms appeared on the inoculated halves about 2 weeks after infection, and on the uninoculated halves 2 to 4 weeks later. Evidence of disease appeared on the noninoculated halves as light green tissue adjacent to the inoculated tissue, or as necrotic rings involving portions of both halves of the leaves.

Fully expanded leaves borne by the youngest bulbs of the plants sometimes develop a transient rather than a lasting symptom. A few days after symptoms appear on the older leaves, the younger inoculated leaves develop irregular, lighter green, ovoid areas which at first can be seen only by transmitted light, and which suggest incipient water-soaked areas. These lesions may progress to form conspicuous yellowish green areas, or may persist for a few weeks and then gradually disappear.

Ring-spot virus produced no evident flower symptoms in the two diseased plants which flowered. These had been infected for 7 months prior to blooming.

The available evidence suggests that ring-spot virus causes an initial shock effect on *Odontoglossum* indicated by the production of striking leaf symptoms, whereas later growth appears to carry the virus with little or no visible evidence of the disease. Whether this masking is permanent or temporary is at present unknown. A year after the initial diseased plant was received, juice from one new bulb and its leaves, which had no definite symptoms, proved infectious when inoculated into healthy plants. The leaves produced during the summer of 1950 on plants experimentally infected during the first week of January 1950 were symptomless and of normal size, but their juice was infectious when inoculated into healthy plants.

ELECTRON MICROSCOPY

In preparation for study of the virus in the electron microscope, leaves taken from diseased and healthy plants were subjected to parallel treat-

ment as follows: 2 cc. of 0.1M phosphate buffer (pH 7.0) was added per gm. of leaf tissue. This was ground in a mortar with the aid of washed quartz sand. The ground suspension was strained through cheesecloth, then the juice was centrifuged at 10,000 r.p.m. for 10 min. The supernatant was centrifuged at 35,000 r.p.m. for 1 hr. The resulting pellet from diseased leaves was transparent, but tinted green by chloroplast fragments; that from the healthy leaves appeared to consist entirely of chloroplast fragments. These pellets were suspended in 0.1M phosphate buffer, pH 7.0, and subjected to two further cycles of low- and high-speed centrifugation. The

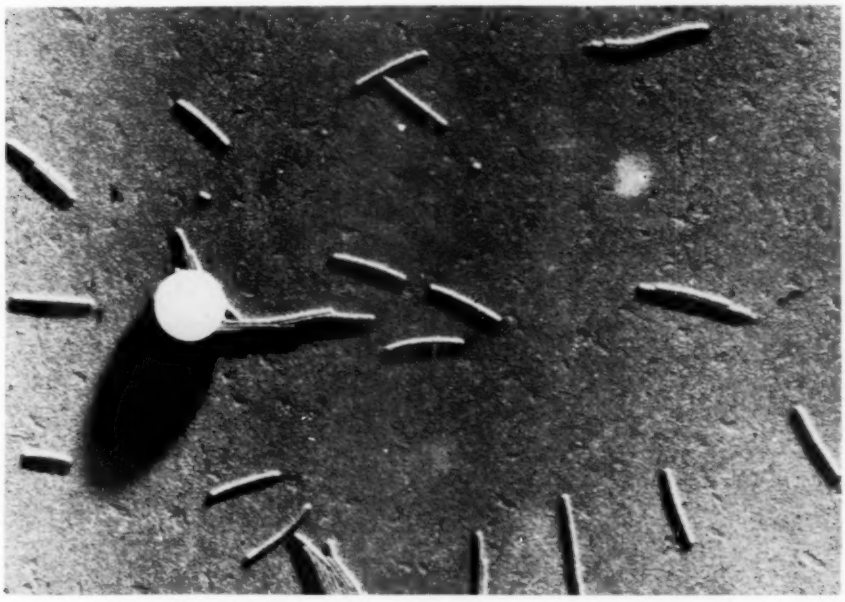

FIG. 2. Electron micrograph of purified ring-spot virus of *Odontoglossum grande*. The polystyrene ball is 260 mμ in diameter.

particles in the preparation from healthy tissue readily sedimented on the low-speed centrifugation, so that there was no remaining pellet at the end of the treatment. The pellet from the diseased leaves, after three cycles of centrifugation, was a clear gel. It was suspended in 0.01M phosphate buffer, pH 7.0, to which a few drops of polystyrene latex (Dow Chemical Co. Lot 10t 508G) were added for size calibration (diameter = 260 mμ). The suspension was sprayed on collodion films on electron microscope specimen screens, was shadowed with palladium, and the preparation was examined in the electron microscope. Similar specimens were prepared from healthy tissue, although no pellet remained after the purification procedure.

The preparation from diseased tissue (Fig. 2) contained rod-shaped particles, slightly sickle-shaped. Based on measurement of approximately 1000 particles, the particle lengths (Fig. 3) varied from less than 50 to more than 600 mμ, but there was a rather sharp central peak at about 280 mμ. The particles were uniformly about 24 mμ in width. No comparable particles were observed in the preparation from healthy leaves.

HOST RANGE

Attempts to find plant species, other than *Odontoglossum grande*, which are susceptible to infection by ring-spot virus have thus far been unsuccessful. Twenty-one species of plants representing 19 genera in 11 families of plants including a Cymbidium orchid were mechanically inoculated with expressed juice from ring-spot diseased plants. No symptoms have been produced to date in any of the plants except *O. grande*. The nonsusceptible plant species tested are: *Cymbidium* hybrid; sugar beet (*Beta vulgaris* L.); China aster (*Callistephus chinensis* Nees); *Zinnia* sp.; lettuce (*Lactuca sativa* L.); globe artichoke (*Cynara scolymus* L.); pak choi (*Brassica chinensis* L.); annual stock (*Matthiola incana* R. Br. var. *annua* Voss);

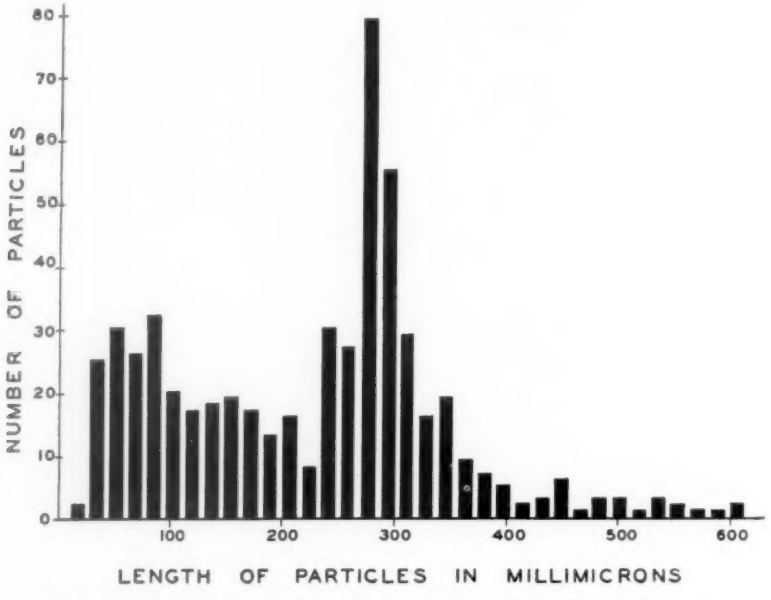

FIG. 3. Length distribution of ring-spot virus of *Odontoglossum*.

squash (*Cucurbita maxima* Duchesne); cucumber (*Cucumis sativus* L.); corn (*Zea mays* L.) var. Golden Bantam; cowpea (*Vigna sinensis* Savi), var. Black Eye; crimson clover (*Trifolium incarnatum* L.); common plantain (*Plantago major* L.); tomato (*Lycopersicon esculentum* Mill.); tobacco (*Nicotiana tabacum* L.) var. Turkish; tobacco (*N. glutinosa*); jimsonweed (*Datura stramonium* L.); garden nasturtium (*Tropaeolum majus* L.); celery (*Apium graveolens* L.) var. Golden Self Blanching.

DISCUSSION

The failure of ring-spot virus to infect nonorchidaceous hosts is not surprising because, of the orchid viruses discussed in the literature, only that infecting *Dendrobium nobile* in Brazil has been reported infectious to species outside of the orchid family. In this latter instance the virus was believed to be a strain of cucumber mosaic virus (7). *Cymbidium* was tested

as a possible host because it shows promise of being susceptible to more orchid viruses than are other genera. In addition to a mosaic virus which it commonly carries, *Cymbidium* is a host of a virus originally from *Cattleya* with particles morphologically very similar to those found in preparations from *Odontoglossum* (Figs. 2, 3).

Of the nonorchidaceous viruses which have been studied in the electron microscope, the particles from *Odontoglossum* appear to resemble most closely the particles of tobacco mosaic virus, but are wider (24 mμ vs. 15 mμ) and possess a stronger tendency toward curvature.

The ultimate effect ring-spot virus has on Odontoglossum plants is as yet unknown. It causes some leaf drop during the early months of infection, but thereafter appears to affect the plant less severely and is possibly carried without symptoms for an indefinite time.

SUMMARY

A virus disease of *Odontoglossum grande* is characterized by single or concentric necrotic rings on the leaves enclosing tissue varying from normal green to black.

Symptoms produced during the first months after infection are more severe than those developing later. New growth produced after infection is symptomless for an indefinite time, but virus can be recovered from symptomless leaves of diseased plants.

Ring-spot virus is readily transmissible to healthy plants by juice inoculation, the first symptoms usually appearing in about 2 weeks.

In the electron microscope, ring-spot virus particles are rod-shaped with a tendency to slight curvature. The particles are uniformly about 24 mμ in width and the most common length is 280 mμ.

The host range of the virus, outside *Odontoglossum*, appears to be limited, as no symptoms were produced in any of 20 species of plants representing 18 genera in 11 families of plants inoculated mechanically with infective juice.

DIVISIONS OF ENTOMOLOGY AND PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

LITERATURE CITED

1. GOLD, A. H. Particles, possibly virus, occurring in orchid. (Abstr.) Phytopath. **40**: 965. 1950.
2. ————, and D. D. JENSEN. An electron microscope study of Cymbidium mosaic virus. Amer. Jour. Bot. (In press.)
3. JENSEN, D. D. Breaking of Cattleya orchid flowers by orchid mosaic virus and its transmission by aphids. Phytopath. **39**: 1056–1059. 1949.
4. ————. Mosaic of Cymbidium orchids. (Abstr.) Phytopath. **40**: 966–967. 1950.
5. ————. Mosaic or black streak disease of Cymbidium orchids. Phytopath. **41**: 401–414. 1951.
6. MURAKISHI, H. H. Color breaking of Vanda orchids. Bull. Pacific Orchid Soc. **9**: 13–16. 1950.
7. NOBREGA, N. R. Uma doenca de virus em orquidea. O. Biologico **13**: 62. 1947.

PHYTOPATHOLOGICAL NOTES

Stem and Root Rot of Daphne odora Caused by Phytophthora parasitica. C. M. Tompkins and C. M. Tucker. *Daphne odora* Thunb., an ornamental flowering shrub grown in nurseries, parks, estates, and home gardens in Santa Clara, San Mateo, and San Francisco counties of California, is susceptible to fungus infection of the stem, crown, and roots during the wet winter season. A similar disease of Daphne (species not given) was found in 1948 in metropolitan gardens at Sydney, New South Wales, Australia,[1, 2] following excessive spring and summer rains. *Phytophthora parasitica* Dastur and *Sclerotium rolfsii* Sacc. were listed as the causal organisms.

Infection of *Daphne* spp. probably is fairly common. In 1937 the writers isolated and identified *P. cactorum* (L. & C.) Schroet. from *D. cneorum* L. at St. Helena, California. In 1938 an isolate of the same species from the same host, obtained by A. W. Dimock at Brewster, New York, was identified. In 1939 J. A. Milbrath sent a culture of the species, also from *D. cneorum* L., obtained in Oregon. Our earliest records of *P. parasitica* on Daphne are in 1940, when J. T. Middleton sent an isolate obtained in California from *D. odora*, and P. A. Miller obtained the species from a crown rot of *Daphne* sp. at Ontario, California. All identifications were by one of the writers.

The principal environmental factors that favor inception and spread of the disease are excessive irrigation, inadequate drainage, and cool weather. The disease is not confined to low, flat areas but may occur on sloping ground if the soil is kept extremely wet. The crop may fail when soils become waterlogged. When Daphne plants are grown on well-drained sites and supplied with only enough moisture to satisfy growth requirements, the incidence of the disease is greatly reduced.

Plants of *Daphne odora* of all sizes, from rooted cuttings to large, flowering specimens, appear to be susceptible to infection. Dark-brown to black, irregular, water-soaked lesions appear first on the stem at or near the soil line and later coalesce to form larger lesions which frequently girdle the stem. Internally, the invaded tissues turn brownish-black and are sharply delimited from the adjoining healthy areas. Discoloration of the stem, both internally and externally, may extend for several centimeters above the soil line, while all crown and underground parts are involved. The water-soaked, blackened roots quickly disintegrate. Coinciding with stem, crown, and root infection, severe wilting of the foliage and flowers occurs. In late stages of the disease, infected plants may collapse because of structural instability.

Diseased material collected at Millbrae, California, in October 1946, and plated on malt-extract agar, has consistently yielded *Phytophthora para-*

[1] Anonymous. Plant disease survey for the twelve-month period ending 30th June. Eighteenth Ann. Rpt., New South Wales Department of Agriculture. 70 pp. 1948.

[2] Anonymous. Plant disease survey for the twelve months ending 30th June, 1949. Nineteenth Ann. Rpt., New South Wales Department of Agriculture. 51 pp. 1949.

sitica Dastur. The isolates grew well on various culture media. On cornmeal agar (pH 6.0) at 25° C., the type of mycelial growth was indistinguishable from that of an isolate of *Phytophthora parasitica* obtained from a tomato fruit in Missouri. Tufts of mycelium in a somewhat concentric arrangement appeared as the fungus spread over the plates. The optimum temperature for growth was 28°–30° C., and all isolates grew fairly rapidly at 35° C.

Oogonia, oospores, and antheridia were not found in cultures on several organic media. Sporangia and chlamydospores were abundant in oatmeal agar cultures 4 weeks old. The sporangia were hyaline, limoniform, conspicuously papillate, 23–62 × 17–48 μ, with a mean size of 47.3 ×34.1 μ. Chlamydospores were spheroidal, with a slightly thickened wall, hyaline to light brown, 16–42 μ in diameter, with a mean of 26.2 μ.

The Daphne isolates behaved similarly, in every respect, to the tomato isolate, and are considered typical of *Phytophthora parasitica*. In wounded apple fruits they caused a firm, somewhat mealy and dry, light brown rot visible as a brown circular area about 1 in. in diameter 4 days after inoculation. In wounded potato tubers, after 8 days, the invaded tissue was slightly flabby and involved one-quarter to one-half of the tubers. The cut surfaces of invaded tissues were not discolored, but after a few minutes' exposure to air, they became pink. When young tomato plants were inoculated in wounds near the base of the stem, a dark rot of the tissues resulted in collapse of the plants. In similar inoculations of pepper and tobacco plants, the isolates proved nonpathogenic.

Two isolates of the fungus proved pathogenic to healthy plants of *Daphne odora* grown in sterile potting soil from cuttings. Inoculum was prepared by growing the fungus on sterilized cracked wheat in 8-in. test tubes. When ready for use, this was added to autoclaved soil contained in 6-in. pots in such a manner as to avoid injury to the root system of the young plants. Sterile cracked wheat was added to the pots serving as controls. All pots were heavily watered each day in order to keep the soil very moist. The incubation period ranged from 3 to 5 weeks, and all infected plants died within 2 weeks after symptoms of the disease were well advanced. Of 14 healthy plants inoculated with each isolate, none escaped infection, whereas the 10 control plants remained healthy. The fungus was reisolated from each infected plant and proved to be identical with the original isolates. The reisolates proved highly pathogenic. Symptoms on the artificially infected plants were identical with those of naturally infected plants.

Twelve young, healthy plants of *Daphne cneorum* artificially inoculated in the greenhouse with one isolate of the fungus became infected and died within 4 weeks after inoculation. Six noninoculated control plants remained healthy.—Division of Plant Pathology, University of California, Berkeley, California, and Department of Botany, University of Missouri, Columbia, Missouri.

Hollow Heart of Cacao Beans. R. CIFERRI. A peculiar malformation of fresh Criollo cacao beans in Venezuela is characterized by blackish-brown discoloration of the central portion of the seed which appears as a small lenticular cavity. This cavity, which is only recognizable in transverse or longitudinal sections of the fresh bean, is completely enclosed by the surrounding healthy, pale pink or white flesh of the cotyledons. The affected tissues surrounding the cavity, and the cavity itself, are always sterile.

Hollow heart is found only in beans of Criollo subspecies or in high-quality hybrids, e.g., the best crosses with well-defined characteristics of Criollo cacao of the Venezuelan Forastero[1] which is the best market-type commercial cacao. Therefore the presence of hollow heart can be used as an indication of the degree of Criollo stock in superior strains of the Venezuelan Forastero.

Beans of both Criollo and native types, having pods with greenish-white or purplish-violet color before maturing, are affected by the disease but it is most frequent in the Venezuelan Criollo type. All pods from a single diseased tree contain abnormal beans but apparently the number of diseased beans is greater in pods that develop during the dry season or in pods of old or unhealthy trees. Beans with hollow heart may be distributed irregularly throughout a pod but most frequently they are localized in the distal portion of the fruit. The disease is recognizable only during the last stage of ripening of the pods. It begins as a narrow, short, brown stripe which increases along both orthogonal axes. Diseased seeds do not develop into seedlings.

Hollow-heart disease of cacao is of no economic importance as it is found in only a very small percentage of beans, rarely reaching 10 to 15 per cent or from 1 to 5 seeds per pod.

The first hypothesis as to the nature of the disease is that it is of genetic origin, but explanation is difficult. An alternative hypothesis is that the disease is the result of a nutritional deficiency such as is frequently found in cacao estates in the sandy valleys of the Caribbean coasts.—Italian Cryptogamic Laboratory and Botanical Institute, The University of Pavia, Italy.

[1] Ciferri, R. Early 'Criollo' cacao in Surinam and the origin of 'Forasteros' of Trinidad and Venezuela. Nature 163: 953. 1949.

MEETING OF THE NORTHEASTERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The Northeastern Division met at Springfield, Massachusetts, on December 19 and 20, 1950. Abstracts of thirteen papers were presented at the meetings.

Control of Tobacco Nematodes by Soil Fumigation. ANDERSON, P. J. Two kinds of nematodes affect Connecticut Valley tobacco: 1) root-knot nematode (*Heterodera marioni*) and 2) the brown root-rot or meadow-nematode group (*Pratylenchus pratensis* and others). Experiments of the last 5 years show that both can be controlled by fumigating the fields with ethylene dibromide or DD. Affected plants are often so stunted that the crop is not worth harvesting. On a lightly infected field replicated tests gave an increase of 11 per cent in yield and further increase in grading of 11 per cent, a net increase of $150 an acre by fumigation. Part of the benefit is due to increase in the available nitrogen in the soil. Bromine content of leaves is increased by ethylene dibromide but has no harmful effect. Taste, aroma, and combustion qualities of the cured tobacco are not affected. Benefits last at least 2 years.

Effectiveness of Three Phenyl Mercury Formulations for the Control of Apple Scab. COHEN, SYLVAN I. Because of variable individual field performance of three phenyl mercury fungicides, a critical study was undertaken to compare these compounds for fungitoxicity under similar conditions. The chemicals studied were phenyl mercuri triethanol ammonium lactate (Puratized Agricultural Spray), phenyl mercuri monoethanol ammonium acetate (Puratized Apple Spray), and phenyl mercuri formamide (Puratized 806). Equimolar aqueous solutions of each compound performed differently in spore-germination tests and in growth-inhibiting concentrations against seven species of bacteria and seven species of fungi. However, solutions adjusted to contain equivalent amounts of the phenyl mercury group for each compound gave significantly similar results in these tests; viz., similar LD-50 values and slopes for *Stemphylium* and *Sclerotinia;* and bacteriostatic and fungistatic concentrations. Common lots of these equivalent phenyl mercury solutions were tested against apple scab at six locations on one or more varieties. Two years' results for six varieties showed consistently equal scab control between formulations, although in one year or at one location occasionally, significant differences were reported. These differences did not recur. It is suggested that the C-Hg-N bond is the active component of these particular water-soluble phenyl mercury compounds associated with toxicity to microorganisms.

Control of Brown Patch of Turf in New Jersey. DAVIS, S. H., R. E. ENGEL, AND G. SILBER. In 1950, nine fungicides were tested for their value in the control of brown patch (*Rhizoctonia solani*) on seaside bent (*Agrostis palustris*). Nine applications were made between June 29 and September 18 on three replicated and randomized plots of 50 sq. ft. for each treatment. Seven times throughout the summer, brown-patch outbreaks were counted and measured. The treatments were ranked thus according to control: Caloclor (mercuric and mercurous chloride) and Orthocide 406 (N-trichloromethylthio tetrahydrophthalimide), less than 1 per cent diseased; Crag 531 (calcium zinc copper cadminate), Puraturf (phenyl mercury triethanol ammonium lactate), P.M.A.S. (phenyl mercury acetate), Tersan (tetramethyl thiuramdisulfide), and Crag 1025 (Cadmium and mercury complex), 4 to 9 per cent; Actidione (antibiotic), and Puraturf 177 (p-amino phenyl cadmium dilactate), 39 and 63 per cent. Caloclor, Puraturf, and P.M.A.S., were definitely toxic to the bent grass in mid-summer. Puraturf and P.M.A.S. eliminated the crab grass in the plots. Two commercial stickers, Spred-O-Stick and VL600, added to Tersan treatments and Spred-O-Stick added to a Puraturf treatment showed that it was impractical to double the length of time between treaments and still control brown patch. When used at the standard 10-day intervals these treatments gave results similar to those of the fungicides when used alone.

Top Soil Application of Chemicals for Controlling Carnation Wilt Diseases. GUBA, EMIL F., AND RALPH W. AMES. Numerous chemicals were compared to determine their effectiveness in disinfesting soil of *Fusarium dianthi, Rhizoctonia solani,* and *Phytomonas caryophylli* and were applied at rates of 0.05, 0.1, 0.2, and 1.0 gm. per 30 gm. of inoculated soil (theoretically 4 sq. in. by ¾ in. or 6, 12, 24, and 120 oz. to 100 sq. ft. of bed). Neither fungus was recovered from any of the mixtures of Phygon and soil. Fermate

was strongly fungicidal and striking action was shown by Semesan Jr., Semesan, Semesan Bel, New Improved Ceresan, and mercurie bichloride at 0.05 gm. Only mercurie bichloride was bactericidal at this concentration. At the 0.1-gm. rate, all of the mercuries were fungicidal and New Improved Ceresan, Pfizer's M mixture, and mercuric bichloride were fungicidal and bactericidal. At this rate, neither fungus could be recovered from the soil mixture. Both Puratized Apple Spray and Tag fungicide in the soil were fungicidal but not bactericidal at dilutions of 1: 800. At dilutions of 1: 400, Puratized Agricultural Spray was neither fungicidal nor bactericidal. In our applied studies we have not controlled Fusarium or bacterial wilt. The control of Rhizoctonia stem rot was best with Fermate, New Improved Ceresan, Semesan, and Pfizer's M Mixture in six applications over a period of 5 months in advance of and during the critical summer season for disease. Flower production was best with New Improved Ceresan and Fermate but the improvement over no treatment was not significant. Tag Fungicide 331 applied four times at 3-wk. intervals gave significant control of Rhizoctonia stem rot.

A Method of Making Artificial Field Inoculations on the Leaves and Fruit of Tomatoes. HOADLEY, A. D. Aluminum foil envelopes were used as individual moisture chambers for field inoculations of tomato fruit with *Colletotrichum phomoides* (Sacc.) Chester and tomato leaves with *Stemphylium solani* Weber. Temperatures sufficiently high to kill or greatly retard the growth of some tomato pathogens would be encountered in a field moisture chamber of nonreflective material. Fluctuations of day and night temperatures induced condensation of moisture on inoculated portions of the plant within the foil envelope. Free moisture was continuously present within the foil moisture chamber. Artificially induced *C. phomoides* and *S. solani* lesions were typical of naturally occurring lesions. Sporulation of artificially induced *S. solani* lesions initiated a rapid spread of this pathogen to other parts of susceptible plants. Since temperature governs the rate at which lesions develop, plants of known susceptibility were used as indicators for making disease-resistance readings. The use of aluminum foil envelopes as field moisture chambers may be applicable to other tomato diseases as well as to diseases of other crops.

Relative Specificity of Sclerotinia and Stemphylium to Toxicants. HORSFALL, JAMES G. The fungitoxicity of some 1,000 organic compounds to *Sclerotinia fructicola* and *Stemphylium sarcinaeforme* has been studied using the spore germination technique. *Sclerotinia* lives in the sugar and acid habitat of the ripening peach. *Stemphylium* prefers old foliage of clover and a higher pH. These differing requirements probably explain the findings, (a) that acid compounds (–COOH, $-SO_3H$, even amine hydrochlorides) inhibit *Stemphylium* the more, (b) that basic $-NH_2$ compounds usually inhibit *Sclerotinia* the more, (c) that hydrazines which can react with sugars are more toxic to *Sclerotinia*. Phenolic hydroxyls are more toxic to *Sclerotinia*. The C = O group is especially toxic to *Sclerotinia*, whether it occurs in a quinone, an ester, or a bridge between two aryl groups. Sometimes, but by no means always, the toxicity of C = O is associated with keto-enol tantomerism. Chlorination seldom confers fungitoxicity (in contrast to insecticides), but chlorination often improves weak fungitoxicants. Apparently, *Sclerotinia* requires a bigger lipophilic group than *Stemphylium*. For example, *p*-phenylene diamine is toxic to *Sclerotinia*, not to *Stemphylium*. α and β Naphthylamines are toxic to both. Phenanthrylamine is toxic to *Sclerotinia*, not to *Stemphylium*.

Mechanical Transmission of Corn-Stunt Virus to An Insect Vector. MARAMOROSCH, KARL. Viruliferous *Dalbulus maidis* DeL. & W. were macerated at 0° C., diluted to 10^{-2} with 0.25 M neutral buffered saline, and centrifuged at 3000 r.p.m. for 10 min., and 1/1000 to 1/8000 cc. of the supernatant injected into each of 100 virus-free *D. maidis*. Single inoculated insects and controls from the same stock were transferred at weekly intervals to healthy corn plants, which were observed in the greenhouse for 3 to 4 months. Of four injected leafhoppers that survived more than 6 weeks, three infected successive corn plants until they died. Controls proved virus-free. The test was repeated twice and mechanical transmission from insect to insect confirmed. In another test a stunted corn leaf was macerated, centrifuged, and diluted in the same manner as the viruliferous insect juices. Out of 120 leafhoppers, each injected with 1/8000 cc. of a 10^{-2} dilution of corn juice, two transmitted virus as long as they lived. Controls proved virus-free. The minimum incubation period of the virus in insects injected with either insect or plant juices was 6 weeks. These experiments add the fourth plant virus to the list of those transmitted to insect vectors by the injection method.

Studies of Hormodendrum resinae Lindau, a Common Inhabitant of Creosoted and Coal-Tar-Treated Wood. MARSDEN, DAVID H. Two distinct morphologic forms of *Hormodendrum resinae* have been isolated from creosoted telephone poles. One isolate (New

York isolate) is brown-spored, the other (Enola isolate) has spores which are olive-gray en masse. Each of these isolates has saltated in culture. Certain physiological studies of the Enola isolate are summarized: On nutrient agar containing creosote the growth rate of the fungus is reduced sharply by concentrations of creosote up to 2 per cent. With greater concentrations up to 8 per cent the growth rate is nearly a constant value. That fraction of creosote which distills between 270° and 315° C. is more inhibitory than other distillate fractions and whole creosote. It appears that *H. resinae* can utilize coal tar and creosote as a N source, the former more readily than the latter material. Where coal tar is the N source, increasing amounts of coal tar up to at least 5 per cent by volume in the medium are reflected in a more nearly normal type of growth and sporulation. Some growth occurs with coal tar as the sole C source, none with creosote. This apparent ability to metabolize these products leads one to suspect that over long periods of time the fungus may degrade their preservative values.

Preliminary Report on Fungicides in Relation to Apple Yield and Quality. PALMITER, D. H., AND R. M. SMOCK. In 1949 long-term fungicide test plots were established in a commercial McIntosh orchard in the Hudson Valley to determine any favorable or adverse effects on yield and quality. The greatest apparent difference the first season was the effect on rate of fruit maturity. All plots were harvested at one time but data on fruit color, firmness, and respiration rate indicated that the fruit ripened in this order: Crag 341-C (2-heptadecyl glyoxalidine), Micronized and flotation sulfur, Phygon (2,3-dichloro-1,4-naphthoquinone), Fermate (ferric dimethyl dithiocarbamate), and Tag (phenyl mercuric acetate). Fermate was substituted for Phygon and Tag in the cover applications. In 1950 the plots were picked in the above order over a 12-day period. Tests on stored fruit indicated about the same state of maturity except in the case of fruit from the Crag plots which were less mature than the others. Other consistent differences noted in the first two years were: best scab control with Tag, Fermate, and Phygon; largest fruit with Tag and Phygon; best fruit color with Fermate, Phygon, and sulfur followed by Fermate; best yield with sulfur, sulfur followed by Fermate, and straight Fermate; and least harvest drop with Tag and sulfur followed by Fermate.

Properties of Tobacco Mosaic Virus Determined by its Inactivation Deuterons. POLLARD, ERNEST, AND A. E. DIMOND. Thin dried films of purified TMV were bombarded with deuterons, after which the virus was dissolved in buffer and assayed for activity by the local-lesion technique. After correcting lesion counts by the count resulting from mere dilution of virus, inactivation caused by deuteron bombardment was determined and plotted as a function of dosage. Inactivation must occur at the site of the primary ionization caused by deuterons in the dried virus itself, since secondary effects occur within a few atomic radii of this point. By varying deuteron energy, one may vary ionization density, the distance by which primary ionizations are separated. When this distance approaches the dimensions of the infective virus particle, one can estimate its size. This proves to be $50 \times 150 \times 2700$ Å. Moreover, TMV presents a target with cross section (the area within which an ionizing event causes inactivation) of 23×10^{-12} cm^2. This remains constant as ionization density is doubled. For a particle having the dimensions given above, these results are explained if inactivation results from a single primary ionization occurring anywhere in the virus. Thus, only one molecular species occurs in the minimum infective particle of TMV. This is in striking contrast to similar experiments on viruses of bacteria where target cross section is proportional to ionization density. Assistance from the Atomic Energy Commission is acknowledged.

Oak Wilt. REX, EDGAR G. Oak wilt, a systemic disease of oak trees caused by the fungus *Chalara quercina* (Henry), has taken a considerable number of trees of the genus *Quercus* in the north-central States. According to artificial inoculations, no species is known to be immune. The species of the black (red) group are far less resistant to the advance of the fungus than are the species of the white oak group. The establishment of new infections beyond the root grafting range is not understood. The fungus readily spreads from a diseased tree to a near-by healthy tree of the same species through root grafts. Intraspecific root grafting has been demonstrated for many species; interspecific root grafting has not. Up to 1950 disease distribution has been surveyed in Wisconsin, Minnesota, Iowa, Missouri, Illinois, and Indiana. During 1950 Arkansas, Ohio, and Pennsylvania were added to this list. Secondary spread from tree to tree can be minimized or prevented by the prompt interruption of the physiological connection afforded by the root grafts. This can be accomplished by 1) mechanical severance of the roots, or 2) chemical injection of the diseased tree so as to clean the involved roots. Twig samples of suspected trees should be submitted to the Department of Plant Pathology of the Agricultural Experiment Station, Trenton, New Jersey, for culturing and diagnosis.

A Three-Year Response of Six Varieties of Tomato to a Fungicide Spray Program. SCHROEDER, W. T., AND W. T. TAPLEY. Sprayed and nonsprayed plots of Gem, Red Jacket, John Baer, Longred, Wisconsin 55, and Rutgers were planted in random order in each of six blocks during the years 1947 thru 1949. A five-application schedule of ziram-ziram-Bordeaux-ziram-Bordeaux was used on the sprayed plots. Yield and disease data obtained over the 3-year period indicate that both were functions of variety and season. Although spraying had no significant effect on total yield of ungraded fruit, it increased the yield of number one equivalent fruit of all varieties. The 3-year average increase ranged from 2 tons per acre for Wisconsin 55 to 7 tons for Rutgers. Increases within individual years ranged to as high as 10 tons per acre. Red Jacket outyielded all other varieties. Field data, supported by greenhouse inoculation tests, showed Longred and Wisconsin 55 to be slightly more tolerant of late blight than the other varieties. Spraying had no significant effect on the development of blossom-end rot, but variety and season did. Anthracnose development was significantly greater in Gem and Wisconsin 55, being almost twice as great as in John Baer and Longred.

Organic Fungicides on Apples in 1950. THURSTON, H. W., JR. A comparison of materials and schedules for the control of apple scab in Pennsylvania in 1950 gave best results with split schedules in which mercury (Tag 331) was used before bloom and either glyoxalidine (Crag 341) or magnetic sulphur in post-bloom sprays. The use of a gloss meter to measure the gloss on fruit at harvest time (one factor in fruit finish) is described. Glossiest fruit (Stayman) as well as least russeted was obtained with the use of mercury followed by glyoxalidine. In a further study of two formulations of glyoxalidine the nature of the dosage-response curves is shown to vary with the test variety, and also to depend on whether the curve is obtained from scab-control data on fruit or on foliage.